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PRINCIPAL INVESTIGATOR: Robert Hanson, Ph.D.

CONTRACTING ORGANIZATION: Northeastern University
Boston, Massachusetts 02115

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6. AUTHOR(S)
Robert Hanson, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Northeastern University
Boston, Massachusetts 02115

E-MAIL:
r.hanson@nunet.neu.edu

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13. ABSTRACT ***(Maximum 200 Words)***

The objective of this project is the development of new chemotherapeutic agents for the treatment of hormone responsive breast cancer using a structure-based, solid-phase synthesis approach. We have successfully linked an activated derivative of estradiol to a solid support and converted it to two different series of estrogen receptor ligands. We have begun the biological evaluation of these compounds and initail results suggest that significant receptor affinity is retained. Conformational studies using NMR and computational methods have provided results that can be used in directing the synthesis of newer estrogen receptor binding agents.

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5.Introduction:

The overall objective of this project is the development of new chemotherapeutic agents for the treatment of hormone responsive breast cancer. Based upon our previous synthetic work and molecular modeling studies we selected the 17α-(substituted phenyl)vinyl estradiols as the lead compounds for our drug development program. We chose a solid-phase synthesis approach to more rapidly generate target compounds for subsequent bioevaluation studies. The review of the results and comparison with molecular conformations, determined by both computational and analytical methods, would provide insight into the interactions of the compounds with the target estrogen receptors. Our approach involved developing the solid phase technology on model compounds and then extending it to our target amino-carboxy phenylvinyl estradiols. Simultaneously, we would be generating expertise with the NMR conformational methods and establishing the requisite receptor and cell proliferation assays. As the new compounds were synthesized, we would then be able to undertake the overall evaluation of their biological and physicochemical properties and to assess the structural contributions to receptor affinity and efficacy. The report describes our initial progress in these areas.

6.Body:

The research proposal described 5 Tasks as part of the Statement of Work. These included 1. Synthesis of polymer-bound aminomethyl- and carboxy-phenylvinyl estradiol, 2. Synthesis, isolation and purification of structure-based libraries, 3. Measurement of biological properties, 4. Assessment of structure-biological activity relationships, and 5. Synthesis, purification, characterization and evaluation of second library of compounds. Of these tasks, only the first three were scheduled for work during the first year of the project and therefore the report will be limited to those tasks.

Task 1. Synthesis of polymer bound 17α-(3-aminomethylphenyl) and 17α-(4-carboxyphenyl)vinyl estradiol.

As described in the proposal, we have used the carboxylated polystyrene resin as our solid support for the steroid scaffold. Although we could prepare this resin in-house by several methods, it was more efficient to purchase it commercially. Our initial approach for synthesis involved attachment of the ethynyl estradiol to the resin and then performing all of the transformations on the resin-bound steroid. Unfortunately this was not efficient and we have done the initial hydrostannation in solution and taken the crude product and attached it directly to the resin in very good overall yields. The subsequent Stille coupling reaction with model substituted phenyl iodides proceeded in satisfactory yields to give products that were isolated, characterized and submitted for biological evaluation. The results of this work are described in the Tetrahedron paper in the Appendix. The technological significance was reported in a conference on High-Throughput Organic Synthesis sponsored by the Cambridge Healthtech Institute (Appendix).

Task 2. Synthesis, Isolation, Purification and Characterization of the Directed Libraries

We have undertaken the extension of the technology to the preparation of the target aminomethyl and carboxyphenyl intermediates and products. The 3-aminomethylphenyl iodide could be directly coupled to the resin-bound stannylvinyl estradiol in reasonable yields. The product was then split and aliquots were acylated with representative groups, e.g., acetyl, propionyl, or benzoyl, and the resultant amides were cleaved from the resin and purified. Each product was characterized and submitted for biological evaluation. In the same manner, methyl 4-iodobenzoate was coupled to the stannylvinyl intermediate in good yields. However, an attempted hydrolysis of the methyl

ester resulted in substantial cleavage of the steroid from the resin. Therefore, we first prepared the trimethylsilylethyl ester of 4-iodobenzoic acid and coupled it to the resin-bound stannylvinyl estradiol. Selective deesterification was then possible with tetrabutylammonium fluoride to give the free para carboxy group. Functionalization of the carboxylic acid with a variety of amines using standard amino acid chemistry is in progress. This will be described in the Ph.D. thesis of Choon Young Lee and in the papers ultimately emanating from her work.

We have undertaken the characterization of the 17 α-(substituted phenyl) vinyl estradiols using NMR methods. Our initial studies focused on two interesting Z-isomers of the products prepared by Ms. Lee. Using a variety of 1D- and 2D-experiments, we were able to assign all of the H-1 and C-13 resonances in the compounds. Subsequently, NOESY spectroscopy allowed us to determine the preferred solution conformations. These conformations, determined by experimental methods, were then compared to molecular conformations determined by computational methods. The results indicated that the solution conformation corresponded to one of the low energy conformers determined by the computational exercise. This provides a validation of the methods we will be using in the structure-activity relationship component of the project. The details of this study are described in the manuscript by Sebag in the Appendix of the report.

Task 3. Measurement of Biological Properties.

During the initial phase of this study we established the essential bioassays that we would use for the project. In conjunction with our other estrogen-related work, we have standardized the binding assay for ligands using the ER-HBD extracted from the BL 21 cells. Using this system we have determined the relative binding affinity (RBA) values for the 3-aminomethyl-and 4-carboxy/carbomethoxy-phenylvinyl estradiol products. The parent amino derivative has and RBA=18-19 (where estradiol =100), suggesting that the presence of the large 3-aminomethylphenylvinyl substituent is remarkably well tolerated at the receptor binding site. The 4-carbomethoxy derivative is also well tolerated (RBA= 18-26), however, the free acid has a much lower affinity (RBA=0.9-1.3). The presence of the polar, hydrophilic acid is probably poorly accommodated in this lipophilic region of the receptor. The RBA values for the amide derivatives of the 3-aminomethylphenylvinyl estradiol were lower than the parent material. Large (t-BOC, benzoyl) and small (acetyl, propionyl) groups both reduced the affinity to the RBA= 1-6 range. This suggests that the basic amino group may be necessary or that there are very severe steric constraints. Derivatives of the 4-carboxyphenylvinyl estradiol have not yet been evaluated.

We have also established the MCF-7 cell proliferation assay. Although not yet employed for the proposed set of derivatives (RBA values are too low to meet the criterion), the assay has been used for related series of estrogens. In those evaluations, we have observed compounds that express either agonism or antagonism to estradiol stimulated cell proliferation. The validation of this assay has been shown by taking those

compounds to the subsequent immature rat uterotrophic growth assay (an in vivo system) in which the agonists have displayed agonist effects and the antagonists have displayed antagonist effects. Therfore, we are confident that we will be able to identify potential candidates for preclinical evaluation by our protocol.

The results of our related work will be submitted for publication while we will be preparing manuscripts for our receptor binding studies later this year.

7. Key Research Accomplishments:

- Development of resin-bound estradiol intermendiates and conversion to derivatized products- library generation step
- NMR studies identify solution conformations for related substituted phenylvinyl estradiols
- Computational studies of substituted phenylvinyl estradiols identify low energy conformers that correspond to solution conformations
- Estrogen Receptor-Hormone Binding Domain (ER-HBD) assays indicate that parent compounds have high binding affinity for the receptor
- Biological evaluation protocol successfully identified candidates for preclinical studies in related series of estrogen agonists/antagonists

8. Reportable Outcomes:

a. Manuscripts, abstracts, presentations

1. Lee, C.Y. and Hanson, R.N. Solid phase synthesis of 17α-E/Z-(X-phenyl)-vinyl estradiols using the Stille coupling reaction. Tetrahedron (2000) 56: 1623-1629
2. Sebag, A.B., Friel. C.J., Hanson, R.N., and Forsyth, D.A. Conformational studies on (17α,20Z)-21-(X-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols using 1D and 2D NMR spectroscopy and GIAO calculations of C-13 shieldings. J. Org. Chem. (submitted)
3. Hanson, R.N. Synthesis of Auger electron-emitting radiopharmaceuticals. Curr. Pharm. Design (2000) 6 (in press)
4. Hanson, R.N. Development of a solid-phase synthesis approach to the preparation of novel steroid-based libraries. High-Throughput Organic Synthesis, Cambridge Healthtec Institute, San Diego, CA, February 9-11, 2000

b. Degree obtained that was supported by the award

1. Lee, Choon Young, Structure activity relationships of estrogen receptor ligands: Synthesis of (17α,20 E/Z)-21-(substituted phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols using solid and solution phase Stille coupling for C-C bond formation. Ph.D. thesis, awarded June 17, 2000.

9.Conclusions:

At this point it appears that the technical aspects of the project are proceeding as anticipated. The intermediate resin-bound (aminomethyl/carboxyphenyl)vinyl estradiols can be prepared and can be converted to their appropriate derivatives. The assays for determining the affinity and efficacy at the estrogen receptor are in place and have provided preliminary data related to the aminomethyl series. NMR and computational studies have provided initial results about the conformation of the compounds which can be used to correlate the substituent effects with the biological activity. Application of these methods in the next year should yield results to guide the generation of the second libraries indicated in the proposal.

10. References:

None.

11. Appendix:

The appendix material is comprised of copies of the two manuscripts, the review article and the flier for the conference on high throughput organic synthesis.

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Tetrahedron 56 (2000) 1623–1629

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Solid Phase Synthesis of 17α-*E*/*Z*-(X-Phenyl)-Vinyl Estradiols Using the Stille Coupling Reaction

Choon Young Lee[†] and Robert N. Hanson[*]

Department of Pharmaceutical Sciences, Bouvé College of Health Sciences, Northeastern University, Boston, MA 02115 USA

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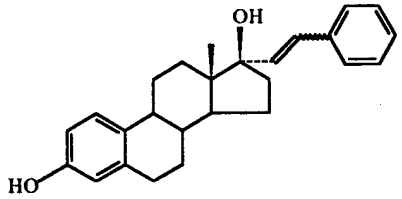
Abstract—As a continuation of our program to develop probes for the hormone binding domain (HBD) of the estrogen receptor (ER), we designed a series of novel 17α-*E*/*Z*-(X-phenyl)-vinyl estradiols. Based upon our experience with solution chemistry we applied solid phase synthesis using carboxylated resins to synthesize the new compounds. The Stille coupling reaction permitted the introduction of a variety of functional groups and positional isomers on the terminal phenyl group. Subsequent cleavage from the resin generated a series of novel estradiol derivatives.

Introduction

As a part of our ongoing program to design and develop new therapeutic agents for the treatment of breast cancer, we have focused on new steroidal derivatives that interact at the hormone binding domain (HBD) of the estrogen receptor (ER). While many of our initial studies confirmed the established estrogen receptor structure activity relationships, derivatives with the *E*- and *Z*-X-vinyl group at the 17α-position particularly demonstrated unusual properties.[1] Further explorations with phenylvinyl (I) and phenylselenovinyl (II) estradiol suggested that receptor affinities comparable to estradiol itself could be maintained in spite of the apparent steric bulk of the 17α substituent.[2] Recent publications of the crystal structure of the liganded HBD of the ER[3] suggested that the 17α groups project into a region that may accommodate significant steric tolerance. We have elected to develop new estradiol derivatives that could exploit that tolerance.



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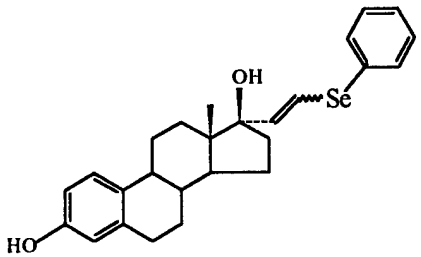

II

The synthesis of our target compounds to date had relied on traditional solution phase chemistry. In order to prepare new derivatives containing a variety of functional groups or existing as positional isomers, we considered approaches that could generate a large number of compounds more easily. The logical choice was solid phase synthesis. We envisioned that we could append our steroid to the inert polymer support, divide it into discrete aliquots, perform the requisite synthetic transformation, remove its individual products from the support and then characterize them. While a significant body of literature existed for solid phase synthesis (SPS) with steroids[4–9] and for Stille coupling,[10–12] there were no prior reports on the specific application that we wished to carry out. For example, Poirer et al., has described solid phase transformations of both androstanes and 16α-substituted estradiols,[4] however, neither employed transformations comparable to those we would require. Similarly, several groups have reported the use of the Stille reaction to couple aromatic and alkyl groups[10–12] but with fewer structural constraints than those imposed by the estrogen scaffold. Therefore, this work involved developing new methods to achieve our objectives.

Keywords: solid phase synthesis; estrogen receptor probes; carboxylation; hydrostannylation; Stille reaction.

* Corresponding author. E-mail: r.hanson@nunet.neu.edu

† E-mail: cholee@lynx.dac.neu.edu

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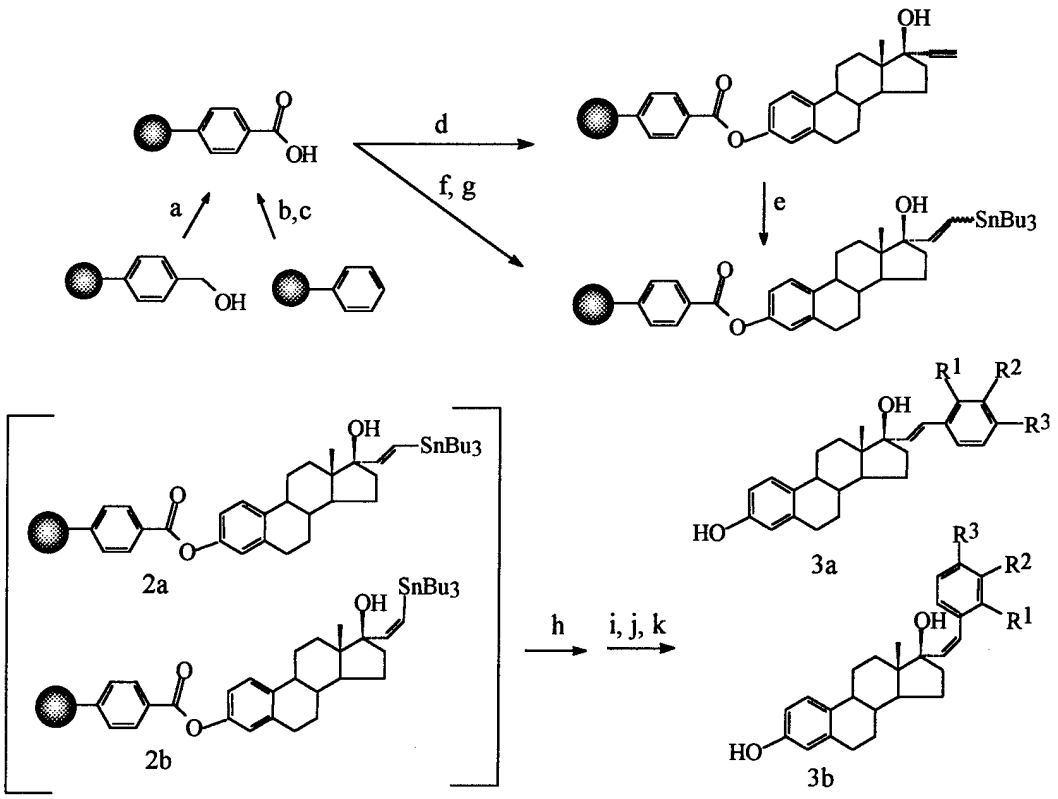


Scheme 1. Reagent: (a) Jones reagent ($H_2Cr_2O_4$, H_2SO_4, acetone); (b) *n*-BuLi, TMEDA, cyclohexane, 50°C; (c) Dry ice, THF; (d) 17α-Ethynyl estradiol, DCC, DMAP, CH_2Cl_2; (e) $HSnBu_3$, Et_3B, THF, 50–60°C; (f) 17α-Ethynyl estradiol, $HSnBu_3$, Et_3B, THF, 50–60°C; (g) DCC, DMAP, CH_2Cl_2; (h) R-Aryl-X, Pd $(PPh_3)_4$, BHT, toluene, N_2, reflux; (i) 5 N-NaOH in CH_3OH–Dioxane (1:3); (j) 5%-CH_3COOH; (k) 10%-$NaHCO_3$.

In this report we demonstrate our approach to developing the solid phase synthesis of the 17α-substituted phenylvinyl estradiols. This involved coupling the steroid intermediates to the resin, identifying appropriate reaction conditions and cleaving the final products from the resin. The result is a reliable method for generating a novel series of functionalized estradiols which can be evaluated for their biological properties.

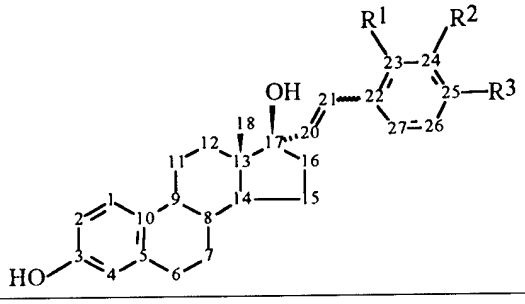
The approach that we selected incorporated several features. First, we chose the carboxylated resins because the estrogen could be selectively coupled through its phenolic linkage to the polymer and the ultimate cleavage of the ester bond at the end of the synthetic sequence would pose few problems. Use of an ether linkage would require either acidic or reductive cleavage, which would not be compatible with the functional groups present in the intermediates or final products. Similarly, amides, carbamates and photolabile links could also present potential problems at various steps of the process. Esterification at the 3-position, however, would not interfere with either the hydrostannylation or the palladium (0) catalyzed coupling reactions that would occur at the 17α-position. The integrity of the tertiary alcohols, *E*/*Z*-styryl groups, or functionality on the terminal phenyl group would be compromised if conditions other than a mild base were used to remove the product from the resin.

Results and Discussion

One of the key elements of the synthetic scheme was the selection of a linker that could be both formed and cleaved under mild conditions. This was based on our observations that 17α-substituted estradiols were unstable under strongly acidic conditions such as those frequently used to release products from the resins. Therefore our resin of choice was carboxylated polystyrene which could be esterified under neutral conditions and ultimately cleaved with mild base. Our first example (compound **8a**) was prepared using the carboxylated resin obtained either by oxidation of a Wang resin using Jones reagent[13] or by carboxylation of a polystyrene resin via lithiation with *n*-butyl lithium.[14] The reactions for both methods were easily monitored by the appearance of the 1700 cm^{-1} absorption in the FT-IR spectrum. The loading capacity of our carboxylated resins was determined by coupling 17α-ethynyl estradiol onto the resins using DCC in the presence of catalytic amount of DMAP and measuring its subsequently cleaved estradiol derivatives from the aliquot of the resins. The loading[15] of oxidized Wang resin was 0.4–0.6 mmol g^{-1} and that of carboxylated polystyrene was 1.5–1.9 mmol g^{-1}. Once we confirmed the utility of coupling through the ester linkage using carboxy polystyrene resin we employed the commercially available carboxy polystyrene for the remainder of our studies. The loading yield of the reaction using the resins with already known loading capacity (2.47 mmol g^{-1}) was 82%. The yield was determined by 'cleave and characterize' methods.

Synthesis of the analogs (Scheme 1) commenced by coupling the 3-phenolic group of 17α-ethynyl estradiol to the carboxy polystyrene resin. An antimony (III) chloride

Table 1. Yields (%) of Stille coupling reaction using solid phase synthesis



Compound	R^1 (*ortho*)	R^2 (*meta*)	R^3 (*para*)	Yield (%)
4a:*E*	CF_3	H	H	38
5a:*E*	H	CF_3	H	33
6a:*E*	H	H	CF_3	49
6b:*Z*	H	H	CF_3	17
7a:*E*	CH_3	H	H	38
8a:*E*	H	CH_3	H	75
8b:*Z*	H	CH_3	H	54
9a:*E*	H	H	OCH_3	36

assay confirmed the presence of the steroids on the resins.[16–18] The absence of color change with bromocresol green suggested that no free carboxylic acid groups remained on the resin.[19] The appearance of a peak at 3301 cm^{-1} in the IR spectrum, corresponding to the C–H stretch of the ethynyl group, also confirmed the reaction and a shift of carbonyl absorption to higher frequency (from 1690 to 1734 cm^{-1}) was also observed.

The subsequent hydrostannylation step incorporated either the use of hydrostannylation of bound ethynyl estradiol (Method A) or hydrostannylation of ethynyl estradiol in solution phase synthesis followed by coupling to the resin (Method B). The resin-bound 17α-ethynyl estradiol was hydrostannylated with tributyltin hydride using triethylborane as a radical initiator[20] to afford a mixture of the 17α-*E*/*Z*-tri-*n*-butylstannylvinyl estradiol in 20–30% (0.12 mmol g^{-1} of *E*, 0.01 mmol g^{-1} of *Z*) loading yields. Varying the reaction conditions, e.g. different solvents, temperatures, or reaction times, did not improve the yields. Therefore, a direct coupling of 17α-*E*/*Z*-tri-*n*-butylstannylvinyl estradiols used to overcome the low efficiency of this step. 17α-Ethynyl estradiol was hydrostannylated at 60°C and the crude mixture was directly transferred to the resin slurry in CH_2Cl_2. The mixture was treated with a 2–3 fold excess of DCC and a catalytic amount of DMAP was added. The loading yield for the coupling reaction was 0.59 mmol g^{-1} with a *Z*/*E* ratio=1:20. The low loading yield was due to use of the acetic acid for the protonation of phenoxide ion after cleavage, subjecting the products to protiodestannylation and reducing the expected loading yield. Because the cleavage after hydrostannylation did not provide a precise loading yield, we subsequently used the dry weight difference between pre- and post-reaction to determine the loading yield. Using the dry weight difference method, the yield for the hydrostannylation reaction was 1.55 mmol g^{-1} for both *E*- and *Z*-isomers. Because hydrostannylation on the resin did not afford satisfactory yields, Method B was the method of choice. As we have previously reported[21] the ratio of *E* and *Z* isomers is a function of the reaction temperature, time and stoichiometric ratio of tributyltin hydride to alkyne. At 60°C the reaction generated greater than 20:1 (*E*/*Z*) ratio bound to the solid phase. To increase the ratio of the *Z*-isomer, triethylborane was used as a radical initiator and the reaction was run at low temperature. The proportion of the *Z*-isomer (*Z*/*E*=1:10) increased, however, the reaction required a longer time and the loading yield for the hydrostannylation was slightly lower than at higher temperature (1.44 mmol g^{-1} by the dry weight difference method) because of more unreacted 17α-ethynyl estradiol in the reaction mixture.

The resin-bound hydrostannnylated estradiol was subjected to the Stille coupling reaction[22] using a variety of substituted aryl halides to generate the target compounds (Table 1). As shown in Scheme 1, $Pd(PPh_3)_4$ was used as the catalyst for the reaction and 3,5-di-*t*-butyl-4-hydroxytoluene (BHT) was added as a scavenger. The use of $Pd(PPh_3)_4$ generated an insoluble by-product that caused coloration of the resin, however, it was easily removed by rinsing it through the built-in filter (50–70 μm). After completion of all the reaction steps, the product was cleaved from the resin by saponification with 5 N NaOH dissolved in CH_3OH–Dioxane (1:3).

As shown in Table 1, the unoptimized yields of the Stille reactions on solid phase ranged from 17–75%, comparable to those observed for solution phase synthesis.[23] Compounds **5a** (*para*-trifluoromethylphenyl, *E*-isomer) and **5b** (*para*-trifluoromethylphenyl, *Z*-isomer) were isolated from the Stille reaction in a ratio of 98:2. Compound **7a** (*meta*-methylphenyl, *E*-isomer) and **7b** (*meta*-methylphenyl, *Z*-isomer) were also obtained in a ratio of 96:4. Although the *Z*-tri-*n*-butylstannyl vinyl estradiol was initially present on the resin, no *Z*-isomers of compound **3a**, **4a**, **6a** or **8a** were isolated from the Stille coupling, instead, 17α-vinyl estradiol, resulting from protiodestannylation was recovered as a side product. Because an excess of reagent was used to drive the reaction to completion, unreacted hydrostannylated 17α-*E*/*Z*-(tri-*n*-butylstannyl)-vinyl estradiol was not detected after the Stille reaction. It is possible that the *Z*-isomers either isomerized to thermodynamically more stable *E*-isomers under the conditions required for the Stille reaction or underwent protiodestannylation. As previously observed, the *Z*-isomer is much more susceptible to protiodestannylation than the *E*-isomer and the appearance of the side product under either solid phase or solution phase synthesis was approximately the same.

The isolated product were characterized by standard spectroscopic methods (FT-IR, ^1H and ^{13}C NMR) and analytical methods. The data were consistent with the proposed structures. Stereochemical assignments for compounds **5a** and **5b** were based on the C_{20}, C_{21} olefinic proton coupling constants for which *E*=16 Hz and *Z*=12.9 Hz, respectively. For compounds **7a** and **7b**, the observed coupling constants were 18.2 Hz for the C_{20} *E*-vinyl proton and 13.1 Hz for the C_{20} *Z*-vinyl proton. In ^{13}C NMR, long range couplings were observed for the compounds **3a**–**5a** and **5b** containing the trifluoromethyl group. Coupling with strongly electronegative fluorine was found at the carbon directly attached to the fluorine ($^1J_{C-F}$) and one ($^2J_{C-F}$) and two carbons distant ($^3J_{C-F}$). The carbons appeared as quartets and the coupling constants

were approximately $^{1}J_{C-F}$=270 Hz, $^{2}J_{C-F}$=32 Hz, $^{3}J_{C-F}$ =3–5 Hz, respectively.

Initial biological evaluation of these compounds indicates that they retain substantial affinity for the ER-LBD (results to be published elsewhere). Because both the properties of the aryl substitutent and its position on the ring (*o/m/p*) appear to affect the receptor binding, a more extensive evaluation of the derivatives is required.

In conclusion, we have applied the Solid Phase Synthesis methodology using carboxylated resins to generate a series of novel ER-LBD ligands. The initial objectives of this study, the simplification of the purification steps and the simultaneous production of both *E*- and *Z*-isomers, were largely achieved. The products were in acceptable yields, however no attempt had been made at this point to optimize conditions and clearly the yields could be improved. Analysis of the products indicated that the initial method provided only the *E*-isomer for most of the target compounds even though both *E* and *Z*-isomers were present after hydrostannylation reaction. We anticipate that modifications in both the coupling and cleavage steps would improve the yields for the chemically more sensitive *Z*-isomers. Nevertheless, this study has demonstrated the feasibility of solid phase synthesis for generating a variety of functionalized estradiol derivatives. Based on our preliminary biological results, we anticipate that further modifications of the phenyl group will yield promising results and we intend to adapt these methods for use in a combinatorial approach to generate diverse target compounds as ER-LBD ligands.

Experimental

Materials

Reagents and solvents were obtained from commercial sources (Aldrich and Sigma) and were used without further purification. Wang resins and carboxylated polystyrene resins were obtained from Novabiochem. The loading capacities of the resins, 0.75 mmol g^{-1} for the Wang resin and 2.47 mmol g^{-1} for the polystyrene resin, were determined by the manufacturer.

General methods

A specially designed flask which had a glass frit, through which the reaction mixture could be filtered by applying pressure, was used for the solid phase synthesis. Purifications for the intermediates were done by rinsing resins three times with the following solvents: CH_2Cl_2, THF, DMF, MeOH, CH_2Cl_2. The cleaved products were purified on a silica gel column chromatography using the appropriate solvents and were characterized by melting point, NMR, IR and elemental analysis. Melting points were determined in open capillary on an Electrothermal Melting Point Apparatus and were uncorrected. IR spectra were recorded on a Perkin–Elmer Model 1600 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained with a Varian XL-300 NMR spectrometer at 300 MHz in $CDCl_3$, acetone-d_6, or DMSO-d_6 as a solvent. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). As on-resin reaction monitoring methods, color tests and FT-IR methods were used. Bomocresol green (0.5% in ethanol, pH=8) was used to assay for free carboxylic acids.[18] The color of the stock solution was dark blue and changed to yellow in the presence of free carboxy groups. Antimony (III) chloride solution (25% in CCl_4) was also used to determine whether the steroid (17α-ethynyl estradiol) was coupled to the resin and a positive test result for the presence of estradiol was indicated by the color purple.[16–18] In addition, a spectroscopic method (FT-IR) was facilitated to detect chromophore change by reaction.

Preparation of the carboxylated resin

(Method A). The Wang resins (1 g, 0.75 mmol) were swelled in the CH_2Cl_2 overnight and rinsed twice with THF, CH_3OH, CH_2Cl_2 and acetone. Acetone (5 mL) was added to the swelled resins. To the slurry was added 1 mL of Jones reagent[13] in a dropwise manner. The mixture was allowed to stand at room temperature for 24 h. The resin mixture was rinsed twice with water–acetone (1:1), CH_3OH, DMF, DMSO and CH_2Cl_2 and dried in vacuo. The loading capacity after the carboxylation reaction was 0.4–0.6 mmol g^{-1}, which was determined with the coupling of 17α-ethynyl estradiol to the resin. The aliquot of the resins was characterized by FT-IR. FT-IR (KBr) ν: 3000–3500 (OH, broad), 1690 (C=O, broad), 1603, 1492, 1452 (aromatic ring), 1279 (C–O).

(Method B). The carboxylation of a polystyrene resin was accomplished using the method described by Farrall et al.[14] FT-IR (KBr) ν: 3420 (OH, broad), 1630 (C=O, broad), 1200–1400 (C–O, broad). Loading capacity: 1.5–1.9 mmol g^{-1}.

Coupling 17α-ethynyl estradiol to the resins

The carboxylated Wang resin (2.3 g) or polystyrene resin (2.5 g) was placed in the reactor equipped with a magnetic stirrer. The resin was swelled in the CH_2Cl_2 for 5 h and washed sequentially with THF, DMF, CH_3OH, THF and CH_2Cl_2. To the resin was added 0.23 g (1.1 mmol) of dicyclohexylcarbodiimide (DCC) and 5 mL of CH_2Cl_2 and the mixture was mildly stirred for 10 min. To the slurry was added 0.75 g (2.6 mmol) of 17α-ethynyl estradiol dissolved in 10 mL of CH_2Cl_2–DMF (9:1) solvent and catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction mixture was stirred for 5 min and then allowed to stand at room temperature for 24 h. The resin was washed three times with CH_2Cl_2, CH_3OH, IPA (60°C), THF and DMF (60°C).[24] The rinsed resin was dried under vacuum for 5 h. The actual loading of the resin was determined by quantitative measurement of the material by cleavage from known weight of resin using 5 N-NaOH in CH_3OH–dioxane (1:3). The resin-bound steroids were characterized by FT-IR and the cleaved compounds by ^{1}H and ^{13}C NMR before proceeding to the next step. The loading capacity of each resin was shown in Method A and B; FT-IR (KBr) ν: 3437 (17β-OH), 3301 (17α-C≡C–H), 1735 (C=O), 1607, 1493, 1452 (aromatic ring), 1216(C–O).

Hydrostannylation

(Method A). The 17α-ethynyl estradiol coupled to the resin (0.49 g, 0.57 mmol g^{-1}) was placed in a dry 25 mL reaction flask equipped with a reflux condenser and a magnetic stirrer and was swelled in THF for 1 h. To the slurry in the dry THF were treated triethylborane (0.7 mL) and tributyltin hydride (1 mL).[20] The mixture was allowed to stand at 60–70°C for 48 h under a nitrogen atmosphere. The reaction mixture was washed three times each with CH_2Cl_2, CH_3OH, DMF, CH_2Cl_2 and ethyl acetate and the resultant resin was dried in vacuo. An aliquot of the resins was cleaved with 5 N NaOH in CH_3OH–CH_2Cl_2 (1:2) to afford a mixture of *E*- and *Z*-isomers. The mixture was separated by chromatography on the silica gel to give a 23% (0.13 mmol g^{-1}) yield of products, consisting of 21% (0.12 mmol g^{-1}) of the *E*-isomer and 2% (0.01 mmol g^{-1}) of the *Z*-isomer. R_f (*Z*-isomer)=0.58 (hexane–ethyl acetate, 4:1); R_f (*E*-isomer)=0.44 (hexane–ethyl acetate, 4:1); Amorphous; ^{1}H NMR ($CDCl_3$, 300 MHz, δ), 0.88 (s, 3H, C_{18}-methyl-H), 1.2–2.4 (m, steroid envelope and tributylstannyl-H), 2.7–2.9 (m, 2H, C_6-H), 6.06 (d, 1H, *J*=19.4 Hz, C_{21} vinyl-H), 6.22 (d, 1H, *J*=19.4 Hz, C_{20} vinyl-H), 6.79 (d, 1H, *J*=2.4 Hz, C_4-H), 6.84 (dd, 1H, *J*=2.6, 8.4 Hz, C_2-H), 7.28 (d, 1H, *J*=8.8 Hz, C_1-H); ^{13}C NMR ($CDCl_3$), 9.6 (C_{22}, 4C), 13.7 (C_{24}, 4C), 14.2 (C_{18}), 23.4 (C_{15}), 26.4 (C_{11}), 27.3 (C_{25}, 4C), 27.4 (C_7), 29.2 (C_{23}, 4C), 29.6 (C_6), 32.4 (C_{12}), 35.9 (C_{16}), 39.4 (C_8), 43.8 (C_9), 46.7 (C_{13}), 49.0 (C_{14}), 85.6 (C_{17}), 112.6 (C_2), 115.2 (C_4), 124.6 (C_{21}), 126.5 (C_1), 132.7 (C_{10}), 138.3 (C_5), 152.4 (C_{20}), 153.3 (C_3); FT-IR (KBr) ν: 3445 (17β-OH, broad), 1719 (C=O), 1653 (C=C), 1607, 1493, 1451 (aromatic ring), 1217 (C–O).

(Method B). The 17α-ethynyl estradiol (3 g, 10 mmol) was dissolved in THF and treated with triethylborane (2 mL, 17 mmol) and tributyltin hydride (3 g, 11 mmol). The mixture was stirred with a magnetic stirrer at 60°C for 16 h. The crude mixture (7.73 g) was evaporated to dryness, redissolved in the CH_2Cl_2, and transferred to the swelled resin (5 g) in CH_2Cl_2 in the presence of DCC. A catalytic amount of DMAP was added to the mixture, which was allowed to stand for 24 h. The resultant functionalized resin was treated as previously described. The total loading for both *E*- and *Z*-isomers was 0.59 mmol g^{-1} with 0.56 mmol g^{-1} of *E*-isomer and 0.03 mmol g^{-1} of *Z*-isomer, however, by the dry weight difference between pre- and post-reaction, the loading for both *E*- and *Z*-isomers was 1.55 mmol g^{-1}.

Electrophilic destannylation on the resin

The Stille reaction was used to couple the anchored *E*- and *Z*-stannylvinyl estradiol to aryl halides. The resin was added to the reaction flask, swelled in the CH_2Cl_2, subsequently treated with 10 mL of anhydrous toluene. To the resultant slurry was added a 3–4 fold excess of the functionalized aryl halide, 1–2 crystals of 3,5-di-*t*-butyl-4-hydroxytoluene (BHT), and $Pd(PPh_3)_4$.[13–15] The reaction was allowed to proceed at 90–100°C for 24 h. After cooling, the resin was washed as previously described, dried in vacuo and weighed.

Cleavage

The resin was swelled in CH_2Cl_2 (10 mL) containing 3 mL of 5 N-NaOH in CH_3OH–Dioxane (1:3), and stirred for 1 h. This cleavage step was repeated three times. Most of the product was collected from the first attempt, a small amount by second hydrolysis and almost none from the third trial. The fractions were combined, evaporated to dryness and partitioned between ethyl acetate and water. Acetic acid (1 mL, 5%) was added. The organic phase was washed with 10% aqueous $NaHCO_3$ to remove the residual acetic acid, dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by silica gel column chromatography or by recrystallization from the appropriate solvent.

17α-20*E*-21-(2-Trifluoromethylphenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (17α-*E*-(2-trifluoro methylphenyl)-vinyl estradiol) (4a). Yield=38%; R_f=0.19 (hexane–ethyl acetate, 4:1); mp 224–225°C; ^{1}H NMR (300 MHz, Acetone-d_6, δ) 1.02 (s, 3H, C_{18} methyl-H), 1.2–2.4 (m, steroid envelope), 2.7–2.9 (m, 2H, C_6-H), 3.98(s, 1H, 17β hydroxyl-H), 6.53 (d, 1H, *J*=2.3 Hz, C_4-H), 6.58 (dd, 1H, *J*=2.6, 8.5 Hz, C_2-H), 6.64 (d, 1H, *J*=15.7 Hz, C_{20} vinyl-H), 7.0 (dd, 1H, *J*=2.5, 15.8 Hz, C_{21} vinyl-H), 7.07 (d, 1H, *J*=8.7 Hz, C_1-H), 7.42 (t, 1H, *J*=7.8 Hz, C_{26}-H), 7.60 (t, 1H, *J*=7.3 Hz, C_{25}-H), 7.69 (d, 1H, *J*=7.8 Hz, C_{27}-H), 7.81 (d, 1H, *J*=8.3 Hz, C_{24}-H), 7.98 (s, C_3 hydroxy-H); ^{13}C NMR (75.4 MHz, Acetone-d_6, δ) 14.7 (C_{18}), 24.1 (C_{15}), 27.2 (C_{11}), 28.3 (C_7), (C_6), 33.4 (C_{12}), 37.5 (C_{16}), 40.7 (C_8), 44.6 (C_9), 48.4 (C_{13}), 50.0 (C_{14}), 84.3 (C_{17}), 113.5 (C_2), 115.9 (C_4), 123.4 (C_{21}), 125.6 (q, *J*=273.2 Hz, C_{28}:CF_3), 126.4 (q, *J*=5.8 Hz, C_{24}), 127.0 (C_1), 127.4 (q, *J*=29.4 Hz, C_{23}), 127.8 (C_{26}), 128.6 (C_{27}), 132.0 (C_{25}), 133.2 (C_{10}), 137.9 (C_{22}), 139.1 (C_5), 142.4 (C_{20}), 155.9 (C_3); Anal. Calcd for $C_{27}H_{29}O_2F_3$: C, 73.30; H, 6.56. Found: C, 73.04; H, 6.68.

17α-20*E*-21-(3-Trifluoromethylphenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (17α-*E*-(3-trifluoro methylphenyl)-vinyl estradiol) (5a). Yield=33%; R_f (*E*-isomer)=0.19 (hexane–ethyl acetate, 4:1); mp 244–246°C; ^{1}H NMR (300 MHz, Acetone-d_6, δ), 1.01 (s, 3H, C_{18}-methyl), 1.2–2.4 (m, steroid envelope), 2.7–2.9 (m, 2H, C_6-H), 3.98 (s, 1H, 17β hydroxyl-H), 6.53 (d, 1H, *J*=2.6 Hz, C_4-H), 6.58 (dd, 1H, *J*=2.6, 8.3 Hz, C_2-H), 6.74 (d, 1H, *J*=16 Hz, C_{21} vinyl-H), 6.84 (d, 1H, *J*=16 Hz, C_{20} vinyl-H), 7.06 (d, 1H, *J*=8.3 Hz, C_1-H), 7.54–7.56 (m, 2H, C_{25}, C_{27}-H), 7.75–7.79 (m, 2H, C_{23}, C_{26}-H), 7.93 (s, C_3-hydroxy-H); ^{13}C NMR (75.4 MHz, Acetone-d_6, δ), 14.7 (C_{18}), 24.1 (C_{15}), 27.3 (C_{11}), 28.3 (C_7), (C_6), 33.5 (C_{12}), 37.5 (C_{16}), 40.7 (C_8), 44.6 (C_9), 48.4 (C_{13}), 50.1 (C_{14}), 84.2 (C_{17}), 113.5 (C_2), 115.9 (C_4), 123.6 (q, *J*=5.6 Hz, C_{25}), 124.1 (q, *J*=3.7 Hz, C_{23}), 125.4 (q, *J*=271 Hz, C_{28}:CF_3), 126.0 (C_{26}), 127.0 (C_1), 130.2 (C_{21}), 130.7 (C_{27}), 131.2 (q, *J*=32 Hz, C_{24}), 132.0 (C_{10}), 138.4 (C_5), 139.7 (C_{20}), 139.9 (C_{22}), 155.9 (C_3); Anal. Calcd for $C_{27}H_{29}O_2F_3$: C, 73.30; H, 6.56. Found: C, 73.42; H, 6.68.

17α-20*E*-21-(4-Trifluoromethylphenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (17α-*E*-(4-trifluoro methylphenyl)-vinyl estradiol) (6a). Yield=49%; R_f= 0.15 (hexane–ethyl acetate, 4:1); mp 215–217°C; ^{1}H

NMR (Acetone-d_6, 300 MHz, δ), 1.02 (s, 3H, C_{18} methyl-H), 1.2–2.4 (m, steroid envelope), 2.7–2.9 (m, 2H, C_6-H), 3.90 (s, 1H, 17β hydroxyl-H), 6.53 (d, 1H, J=2.6 Hz, C_4-H), 6.58 (dd, 1H, J=2.6, 8.4 Hz, C_2-H), 6.73 (d, 1H, J=16 Hz, C_{21} vinyl-H), 6.85 (d, 1H, J=16 Hz, C_{20} vinyl-H), 7.07 (d, 1H, J=8.3 Hz, C_1-H), 7.64 (d, 2H, J=8.7 Hz, C_{23}, C_{27}-H), 7.70 (d, 2H, J=8.6 Hz, C_{24}, C_{26}-H), 8.0 (s, C_3-hydroxy-H); ^{13}C NMR (75.4 MHz, Acetone-d_6, δ) 14.7 (C_{18}), 24.1 (C_{15}), 27.3 (C_{11}), 28.3 (C_7), (C_6), 33.5 (C_{12}), 37.6 (C_{16}), 40.7 (C_8), 44.6 (C_9), 48.5 (C_{13}), 50.2 (C_{14}), 84.2 (C_{17}), 113.5 (C_2), 115.9 (C_4), 125.4 (q, J=270.6 Hz, C_{28}:CF_3), 126.0 (C_{21}), 126.2 (q, J=3.5 Hz, C_{26}), 126.2 (q, J=3.5 Hz, C_{24}), 127.0 (C_1), 127.6 (C_{23}, C_{27}), 128.9 (q, J=32 Hz, C_{25}), 132.0 (C_{10}), 138.4 (C_5), 140.6 (C_{20}), 142.7 (C_{22}), 155.9 (C_3); Anal. Calcd for $C_{27}H_{29}O_2F_3$: C, 73.30; H, 6.56. Found: C, 73.36; H, 6.79.

17α-20*Z*-21-(4-Trifluoromethylphenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (17α-*Z*-(4-trifluoro methylphenyl)-vinyl estradiol) (6b). Yield=17%; R_f= 0.29 (hexane–ethyl acetate, 4:1); ^{1}H NMR (300 MHz, Acetone-d_6, δ) 0.97 (s, 3H, C_{18} methyl-H), 1.2–2.4 (m, steroid envelope), 2.7–2.9 (m, 2H, C_6-H), 3.89 (s, 1H, 17β hydroxyl-H), 6.12 (d, 1H, J=12.9 Hz, C_{21} vinyl-H), 6.48–6.62 (m, 3H, C_2, C $_4$, C_{20} vinyl-H), 7.11 (d, 1H, J=8.1 Hz, C_1-H), 7.59 (d, 2H, J=8.4 Hz, C_{23}, C_{27}-H), 7.80 (d, 2H, J=8.4 Hz, C_{24}, C_{26}-H), 7.95 (s, C_3 hydroxy-H).

17α-20*E*-21-(2-Methylphenyl)-19-norpregna-1,3,5(10), 20-tetraene-3,17β-diol (17α-*E*-(2-methylphenyl)-vinyl estradiol) (7a). Yield=38%; R_f=0.18 (hexane–acetone, 4:1); mp 199–200°C; ^{1}H NMR (Acetone-d_6, 300 MHz, δ), 1.01 (s, 3H, C_{18} methyl-H), 1.2–2.4 (steroid envelope), 2.34 (s, 3H, C_{28} methyl-H), 2.7–2.9 (m, 2H, C_6-H), 3.84 (s, 1H, 17β hydroxyl-H), 6.44 (d, 1H, J=16 Hz, C_{21} vinyl-H), 6.52–6.63 (m, 2H, C_2, C_4-H), 6.83 (d, 1H, J=16 Hz, C_{20} vinyl-H), 7.07 (d, 1H, J=8.3 Hz, C_1-H), 7.10–7.15 (m, 3H, C_{24}, C_{25}, C_{26}-H), 7.48 (d, 1H, J=6.8 Hz, C_{27}-H), 7.97 (s, C_3 hydroxy-H); ^{13}C NMR (75.4 MHz, Acetone-d_6, δ) 14.7 (C_{18}), 19.9 (C_{28}: methyl), 24.1 (C_{15}), 27.3 (C_{11}), 28.3 (C_7), (C_6), 33.5 (C_{12}), 37.5 (C_{16}), 40.7 (C_8), 44.7 (C_9), 48.2 (C_{13}), 50.1 (C_{14}), 84.2 (C_{17}), 113.5 (C_2), 115.9 (C_4), 125.4(C_{26}), 126.5 (C_{25}), 126.9 (C_{24}), 127.0 (C_1), 127.7 (C_{21}), 130.8 (C_{27}), 132.0 (C_{10}), 135.9 (C_{20}), 137.9 (C_{22}), 138.4 (C_5), 138.8 (C_{23}), 155.9 (C_3); Anal. Calcd for $C_{27}H_{32}O_2$: C, 83.51; H, 8.25. Found: C, 83.79; H, 8.65.

17α-20*E*-21-(3-Methylphenyl)-19-norpregna-1,3,5(10), 20-tetraene-3,17β-diol (17α-*E*-(3-methylphenyl)-vinyl estradiol) (8a). Yield=75%; R_f=0.17 (hexane–acetone, 4:1); mp 204–205°C; ^{1}H NMR (300 MHz, Acetone-d_6, δ), 1.00 (s, 3H, C_{18} methyl-H), 1.2–2.4 (m, steroid envelope), 2.31 (s, 3H, C_{28} methyl-H), 2.7–2.9 (m, 2H, C_6-H), 3.74 (s, 1H, 17β hydroxyl-H), 6.52–6.63 (m, 4H, C_4, C_2, C_{21} vinyl, C_{20} vinyl-H), 7.03 (d, 1H, J=7.3 Hz, C_{25}-H), 7.07 (d, 1H, J=8.7 Hz, C_1-H), 7.16–7.31 (m, 3H, J=7.4 Hz, C_{23}, C_{26}, C_{27}-H), 7.93 (s, 1H, C_3 hydroxy-H); ^{13}C NMR (75.4 MHz, Acetone-d_6, δ) 14.8 (C_{18}), 21.4 (C_{28}: methyl), 24.1 (C_{15}), 27.3 (C_{11}), 28.4 (C_7), (C_6), 33.5 (C_{12}), 37.4 (C_{16}), 40.8 (C_8), 44.7 (C_9), 48.3 (C_{13}), 50.2 (C_{14}), 84.2 (C_{17}), 113.6 (C_2), 116.0 (C_4), 124.4 (C_{27}), 127.0 (C_1), 127.7 (C_{25}), 127.8 (C_{26}), 128.5 (C_{21}), 129.2 (C_{23}), 132.2 (C_{10}), 137.0 (C_{20}), 138.4 (C_5), 138.7 (C_{22}, C_{24}), 155.9 (C_3); Anal. Calcd for $C_{27}H_{32}O_2$: C, 83.51; H, 8.25. Found: C, 83.23; H, 8.42.

17α-20*Z*-21-(3-Methylphenyl)-19-norpregna-1,3,5(10), 20-tetraene-3,17β-diol (17α-*Z*-(3-methylphenyl)-vinyl estradiol) (8b). Yield=54% (0.01 g); R_f=0.25 (hexane–acetone, 4:1); ^{1}H NMR (300 MHz, Acetone-d_6, δ) 0.95 (s, 3H, C_{18} methyl-H), 1.2–2.4 (m, steroid envelope), 2.31 (s, 3H, C_{28} methyl-H), 2.7–2.9 (m, 2H, C_6-H), 3.27 (s, 1H, 17β hydroxyl-H), 5.96 (d, 1H, J=13.1 Hz, C_{21} vinyl-H), 6.44 (d, 1H, J=13.1 Hz, C_{20} vinyl-H), 6.53 (d, 1H, J=2.6 Hz, C_4-H), 6.60 (dd, 1H, J=2.6, 8.3 Hz, C_2-H), 7.03 (d, 1H, J=7.3 Hz, C_{25}-H), 7.11 (d, 1H, J=8.3 Hz, C_1-H), 7.17 (t, 1H, J=7.6 Hz, C_{26}-H), 7.38–7.43 (m, 2H, C_{23}, C_{27}-H), 7.95 (s, 1H, C_3 hydroxy-H); ^{13}C NMR (75.4 MHz, Acetone-d_6, δ) 14.58 (C_{18}), 21.42 (C_{28}:methyl), 23.85 (C_{15}), 27.40 (C_{11}), 28.30 (C_7), (C_6), 32.97 (C_{12}), 38.4 (C_{16}), 40.9 (C_8), 44.7 (C_9), 48.8 (C_{13}), 50.1 (C_{14}), 84.3 (C_{17}), 113.6 (C_2), 116.0 (C_4), 127.1 (C_1), 127.8 (C_{27}), 128.1 (C_{25}), 128.3 (C_{26}), 129.7 (C_{21}), 131.4 (C_{23}), 132.0 (C_{10}), 137.1 (C_{20}), 137.6 (C_{24}), 138.45 (C_5) 138.5 (C_{22}), 155.9 (C_3); Anal. Calcd for $C_{29}H_{36}O_3$: C, 80.55; H, 8.33. Found: C, 80.00; H, 8.41.

17α-20*E*-21-(4-Methoxyphenyl)-19-norpregna-1,3,5,(10), 20-tetraene-3,17β-diol (17α-*E*-(4-methoxyphenyl)-vinyl estradiol) (9a). Yield=36%; R_f=0.23 ($CHCl_3$–CH_3OH, 99:1); ^{1}H NMR (300 MHz, Acetone-d_6, δ) 0.99 (s, 3H, C_{18} methyl-H), 3.68 (s, 1H, 17β hydroxy-H), 3.78 (s, 3H, C_{28}:methoxy-H), 6.46 (d, 1H, J=16.1 Hz, C_{21}-H), 6.51–6.59 (m, 3H, C_2, C_4, C_{20}-H), 6.88 (d, 2H, J=8.8 Hz, C_{24}, C_{26}-H); 7.07 (d, 1H, J=8.3 Hz, C_1-H); 7.39 (d, 2H, J=8.8 Hz, C_{23}, C_{27}-H), 7.95 (s, 1H, C_3 hydroxy-H); ^{13}C NMR (75.4 MHz, Acetone-d_6, δ) 14.7 (C_{18}), 24.1 (C_{15}), 27.3 (C_{11}), 28.3 (C_7), (C_6), 33.4 (C_{12}), 37.3 (C_{16}), 40.7 (C_8), 44.7 (C_9), 48.2 (C_{13}), 50.0 (C_{14}), 55.5 (C_{28}:methoxy), 84.1 (C_{17}), 113.5 (C_2), 114.7 (C_{24}, C_{26}), 115.9 (C_4), 127.0 (C_1), 127.0 (C_{21}), 128.3 (C_{23}, C_{27}), 131.4 (C_{22}), 132.1 (C_{10}), 134.9 (C_{20}), 138.4 (C_5), 155.9 (C_3), 159.9 (C_{25}).

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Conformational Studies on (17α, 20Z)-21-(X-Phenyl)-19-Norpregna-1,3,5(10),20-Tetraene-3, 17β-diols Using 1D and 2D NMR Spectroscopy and GIAO Calculations of ^{13}C Shieldings.

Albert B. Sebag, Carolyn J. Friel, Robert N. Hanson*, David A. Forsyth

Departments of Pharmaceutical Science and Chemistry, Northeastern University, 360 Huntington Avenue, Boston, MA 02115

Abstract: Differences in agonist responses of the novel estrogen receptor ligands, (17α, 20Z)-(p-methoxyphenyl)vinyl estradiol (**1**), (17α, 20 Z)-(o-α, α, α-trifluoromethylphenyl)vinyl estradiol (**2**), and (17α, 20Z)-(o-hydroxymethylphenyl)vinyl estradiol (**3**) led us to investigate their solution conformation. In competitive binding assay studies, we observed that several phenyl substituted (17α, 20 E/Z)-(X-phenyl)vinyl estradiols exhibited significant estrogen receptor binding, but with variation (RBA (**1**) = 20; RBA (**2**) = 23; RBA (**3**) = 140 where estradiol RBA = 100) depending on the phenyl substitution pattern. Because the 17α-phenylvinyl substituent interacts with the key helix-12 of the ligand binding domain (LBD), we considered that differences in the preferred conformation of **1**, **2**, and **3** could account for their varying binding affinity. DEPT and two-dimensional COSY, HMBC, and HMQC experiments at 500 MHz allowed the complete assignment of the ^{13}C and ^{1}H spectra of **1**, **2**, and **3**. The conformations of these compounds in solution were established by 2D and 1D NOESY spectroscopy. A statistical approach of evaluating contributing conformers of **1**, **2**, and **3** from predicted ^{13}C shifts correlated quite well with the NOE data. The 17α substituents of **1** and **2** exist in similar conformational equilibria between extended and anti orthogonal geometries with some

differences in relative populations of conformers. In contrast, the 17α substituent of **3**, exists in a conformational equilibrium that includes an anti orthogonal orientation. The similarity in solution conformations of **1** and **2** suggests they occupy a similar receptor volume, consistent with similar RBA values of 20 and 23. Conversely, the different conformational equilibria of **3** may contribute to the significant binding affinity (RBA = 140) of this ligand.

Introduction

Breast cancer is the most common form of cancer among women in the United States with approximately 181,000 new cases diagnosed annually.[1] It is estimated that one in eight women will develop breast cancer during their lifetime and one in three of those will die from the disease. Among the newly diagnosed cases, about 60% are classified as hormone responsive, defined as containing a minimal level of estrogen receptor (ER) and requiring the presence of circulating estrogen to maintain tumor growth.[2] As part of our program to develop more effective therapeutic agents for the treatment of breast cancer, we undertook the designing of new compounds that can potently and selectively block the interaction of estradiol with its target receptor.

Our synthetic efforts have focused on the 17α position as the site for introducing substituents that would impart the desired biological properties. Unlike previous studies with 17α alkyl, aryl or alkynyl groups, suggesting that substituents larger than propyl or propynyl were poorly tolerated[3], we found that the 17α X-vinyl estradiols could bind quite well to the estrogen receptor.[4] Even large substituents, where X = C_6H_5, SeC_6H_5 or SC_6H_5, exhibited significant relative binding affinities (RBA) for the receptor.[5] These observations led us to pursue the synthesis and evaluation of (17α, 20Z)-21-(X-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols (referred to herein as phenylvinyl estradiols) as probes for the estrogen receptor, the results of which are reported in detail elsewhere.[6]

We observed that several phenyl substituted (17α, 20 E/Z)-(X-phenyl)vinyl estradiols exhibited significant estrogen receptor binding (RBA $\geq$ 20 where estradiol RBA = 100 at 2°C), but with variation depending on the phenyl substitution pattern (Figure 1).

(17α, 20 Z)-(p-methoxyphenyl)vinyl estradiol (**1**), for example, exhibited modest agonist responses in vitro and in vivo and shows an RBA of 20 in vitro, while (17α, 20 Z)-(o-α,α,α-trifluoromethylphenyl)vinyl estradiol **2** was similarly potent with an RBA of 23 in vitro. In stark contrast, (17α, 20 Z)-(o-hydroxymethylphenyl)vinyl estradiol (**3**) exhibited significant agonist responses with an RBA of 140 giving **3** more potent estrogen binding affinity than estradiol itself.

Since the placement of a substituent in the *ortho* or *para* positions could affect the conformation, and since the conformational characteristics of 17α-phenylvinyl steroids had not been studied previously, we undertook an investigation of the solution conformation of **1**, **2**, and **3**. Understanding the preferred conformations is one aspect of an effort to correlate the distinctive biological responses derived from these new probes with their structures and ultimately to associate the responses with the ligand-receptor interactions.

The key conformational feature to establish for **1**, **2**, and **3** is the positioning of the 17α side chain relative to the steroid skeleton. The conformation of the relatively rigid steroidal skeleton has been established previously by NMR and other methods. In this study, we use molecular mechanics calculations to generate a set of possible conformations. Two types of NMR data are used in conjunction with the predicted conformations to evaluate which conformations are populated in solution. One approach is to use ^{13}C chemical shifts in a comparison with shifts predicted for each of the geometries generated from the molecular mechanics calculations. The predicted ^{13}C shifts come from empirically scaled GIAO (gauge including atomic orbitals) shielding calculations. The other approach is to compare ^{1}H-^{1}H nuclear Overhauser effects established in one- and two- dimensional experiments, 1D and 2D NOESY, with predicted interatomic distances.

NMR Assignments

Before NMR data could be used to evaluate the conformations of **1**, **2**, and **3**, accurate ^{1}H and ^{13}C chemical shift assignments were required. The one dimensional ^{1}H spectra of **1**, **2**, and **3** in acetone-d_6 (Figure 2(a), 3(a), and 4(a)) reveal that even at 500 MHz, the low frequency spectral regions (1.2-2.5 ppm) are unassignable directly, due to the numerous overlapping signals of the 13 protons resonating in this region. In seeking further separation of the low frequency region, other deuterated solvents were used, namely, benzene, benzene/ acetone, chloroform, chloroform/ acetone, and methylene chloride, but pure acetone provides the best separation. Resonances in the low frequency region that could be readily assigned were the 6α, 6β benzylic protons near 2.8 ppm and the C18 methyl ^{1}H signal at 0.9 ppm.[7] Prior literature reports on ^{1}H NMR assignments of estradiol and other steroids are in disagreement and were of little assistance in assigning the remaining low frequency region.[8] No publication of ^{1}H spectral assignments for any 17α -vinyl substituted estradiols exists.

The most efficient route to ^{1}H signal assignment was to first assign the ^{13}C spectrum. For **1**, **2**, and **3**, the ^{13}C experimental shift assignments were based on the study by Dionne and Poirier on ^{13}C assignments of 17α-substituted estradiols, and our own DEPT and HMBC experiments.[9] The ^{13}C shift assignments were further supported by theoretical shielding calculations (see below). A heteronuclear multiple quantum coherence (HMQC) experiment was performed to correlate proton signals with directly attached carbons. Because the ^{1}H chemical shift assignments derived from the HMQC experiment depended on the accuracy of the ^{13}C chemical shift assignments, other 2D experiments were performed to provide independent evidence. Homonuclear correlation

spectroscopy (H, H- COSY) experiments were performed to correlate the assigned ^1H connectivities. The COSY cross peaks confirmed the initial assignments made by the HMQC experiment. Starting with the unambiguous benzylic H6 signal at 2.8 ppm, the ^1H assignments of the entire aliphatic regions of **1**, **2**, and **3** were confirmed.

The HMQC and H,H-COSY experiments clearly indicated the sites of attachment of all the protons but did not distinguish between the α and β position of the methylene protons. This distinction was readily achieved by using 2D and 1D nuclear Overhauser effect spectroscopy (NOESY) experiments and by comparing coupling constants. Inspection of the ^1H NMR spectrum allows the axial protons, 7α and 6β, to be identified by their larger vicinal coupling constants. The equatorial proton, 11α, is assigned to the isolated signal around 2.4 ppm based on its small coupling constants. The remaining β protons were assigned by the determination of transient NOEs using a 1D NOESY experiment, the 1D analogue of the 2D NOESY experiment.[10] The 1D NOESY experiment avoided problems associated with imperfect subtraction in NOE difference experiments.[11]

Using a selective Gaussian pulse, irradiation of the C18 methyl peaks of **1**, **2**, and **3** gave signal enhancements for the β-protons at positions 8, 11, 12, 15, and 16 (Figure 2(b), 3(b), and 4(b)). These experiments were crucial in making chemical shift assignments, since they resolved β protons from overlapping regions containing α protons. For example, the spectrum of **2** shows a set of four overlapping protons at δ 1.65-1.8; for 12α, 12β, H14 and 15α. Irradiation of the C18 methyl, in the 1D NOESY experiment, reveals at 1.75 ppm the expected 12β signal from the overlapping region. The remaining

assignments in this set are based on the HMQC of steroid **2** that shows that the H14 and 15α protons are slightly further upfield (1.7 ppm and 1.72 ppm) than 12α or 12β. The remaining signal at 1.77 ppm can therefore be assigned to 12α. Assignments in the B and C ring were validated by other 1D NOESY experiments, including the irradiation of H1 that results in the expected enhancement of 11α, and the irradiation of H6, yielding the expected 7α, 7β, and H8 enhancements. In summary, consideration of all the independent NMR experiments allowed the unambiguous assignment of all ^{1}H and ^{13}C resonances. Table 1 summarizes all of the ^{1}H and ^{13}C chemical shifts for **1**, **2**, and **3**.

Theoretical Carbon Chemical Shifts and Solution Conformations

The predicted low energy conformers of **1**, **2**, and **3** were generated using the MM3 force field and were initially determined by rotation around dihedrals C13-C17-C20-C21 and C20-C21-C22-C23 (Figure 5-7).[12] The OH and OCH_3 groups were then rotated so as to find the lowest energy position. For **3**, hydrogen bonding between the 17-OH and 23-CH_2OH group resulted in three pairs (**3a/3c**, **3b/3d**, **3e/3f**) of proton donor/ acceptor conformers. The key dihedral angles for the lowest energy conformers, **1a-1e**, **2a-2f**, and **3a-3h**, with energies within 6 kcal of the lowest energy conformer for **1**, **2**, and **3**, are listed in Table 2. Conformers **1d**, **2d**, **3e**, and **3f**, which have an orthogonal alignment between the estradiol skeleton and the 17α substituent and an anti alignment between the phenyl ring and the C18 methyl, are referred to herein as anti orthogonal conformers. Conversely, conformers **1a**, **2a**, **3a**, and **3c** will be refered to as syn orthogonal conformers. Conformers **1b**, **2b**, **2c**, **3b**, **3d**, and **3h** are designated as extended conformers. All other conformers will be described via a combination of these names.

As the MM3 calculations show, significant changes in the 17α side chain conformation result in minor energy differences. In fact, most of the low energy conformers are within 3 kcal of the lowest energy conformer. This made any conformational determination based purely on energy predictions unreliable.

More reliable conclusions regarding the 17α side chain conformation of **1**, **2**, and **3** could be achieved by comparing predicted ^{13}C chemical shifts for each MM3 conformer to experimental shifts. These predicted ^{13}C chemical shifts, δ_{pred}, were calculated by empirically scaling GIAO-calculated absolute shieldings, σ.[13] The appropriate scaling equation depends upon the basis set. In this study, in which GIAO shielding calculations were obtained at the B3LYP/3-21G level with heteroatoms augmented at the 6-31+G* level, the appropriate scaling is given by eq. (1), as determined previously.[14]

$$\delta_{pred} = -1.168\sigma + 230.2 \qquad (1)$$

All calculations were carried out with the Gaussian 98 program.[15] Tables 3, 4, and 5 list the predicted ^{13}C chemical shifts of each MM3 conformer and the assigned experimental ^{13}C chemical shifts for **1**, **2**, and **3**.

Previously, Dionne and Poirier showed that the carbons in the A, B, and C ring experience little shielding or deshielding effects from various 17α substituents since these carbons exhibit minor (~1 ppm) chemical shift changes. On the other hand, carbons in the D ring were significantly influenced by various 17α substituents. Specifically, C16 and C17 were shown to be the most heavily influenced. Our predicted ^{13}C chemical shifts correspond quite well with the carbons in rings A, B, and C (C1-C14), in fact, most of the

^{13}C predictions in rings A, B, and C are within 1 ppm of the assigned experimental values. These results demonstrate the accuracy of these predictions in an area of a well-defined geometry without any conformational distinction. The shielding and deshielding effects of the 17α substituent are clearly evident in the predicted chemical shift of C16 in different conformers of **1**. In conformers **1b** and **1e**, respectively the second lowest and the highest energy conformers of **1**, the predicted shifts of C16 differ by more than 8 ppm from the experimental value. Similarly for **2** and **3**, the predicted ^{13}C chemical shifts of C16 differ from the observed shift by more than 4 ppm for conformer **2d** and 5 ppm for conformers **2b**, **3f**, and **3h**. These large differences of the predicted shifts of C16 among similar conformers are attributed to the steric interactions between the ortho protons H23/27 and 16α. For example, the predicted C16 shift for extended conformer **1b** with a spatial distance between H23/27 and 16α of 2.2 Å differs from experiment by more than 8 ppm while the C16 shift prediction for anti orthogonal/ extended conformer **1c** with a distance between H23/27 and 16α of 3.2 Å is within one ppm of the experimental value.

If **1**, **2**, and **3** are rapidly exchanging among conformers, only average positions of the ^{13}C resonances will be observed experimentally on the NMR time scale. To determine the contributing conformers of **1**, **2**, and **3**, we chose a statistical approach in which the predicted ^{13}C shifts of the C and D rings of all reasonable conformers of **1**, **2**, and **3** were in each separate case treated as independent variables in a multiple independent variable regression analysis of the corresponding experimental data.[16] The predicted ^{13}C shifts of the A and B rings of all reasonable conformers of **1**, **2**, and **3** were not used in this statistical analysis since they are all within 1 ppm of the experimental values regardless of the conformer. The regression analysis yielded fractional populations as the fitting

parameters. All standard errors and confidence levels of the regression analysis were estimated using the Bootstrapping method.[17] The results given in Table 6 indicate that **1** and **2** each have a major conformer, **1c** (68%) and **2c** (60%). Two minor conformers are also indicated for each: **1d** (12%) and **1a** (20%), and **2a** (20%) and **2f** (20%). For **3**, conformers **3a** (36%), **3d** (34%), and **3e** (28%) were found to be similarly populated with a very minor contribution of **3h** (2%).

NOESY Studies

The solution state conformations of the 17α side chain of **1**, **2**, and **3** were also probed by 2D and 1D NOESY experiments. In the case of **1**, the low frequency region of the 2D NOESY spectrum reveals strong cross peaks involving the vinyl proton, H20, with H14 and 12α,β. A weaker cross peak with 16α could also be detected. The 2D NOESY spectrum also reveals weak cross peaks between the H23/27 aryl protons and four alkyl protons, 12α, 12β, 16α, and 16β. The NOE data provide evidence for more than one conformer since no single conformer of **1** is expected to have an NOE with either H23 or H27 and both 12α and 16α. As all the predicted low energy conformers of **1** show, structures with a distance between H23 or H27 and 12α appropriate for an NOE preclude an NOE with 16α due to too great of a distance (>5 Å). Conformer **1c**, for example, which has a distance between H27 and 16α of 3.2 Å, has a distance greater than 5 Å between H23 or H27 and 12α.

In keeping with the 2D NOESY results for **1**, the selective 1D NOESY of H20 reveals equally strong enhancements of 12α,β and H14 and a weak enhancement of 16α (Figure 2(c)). Similarly, the 1D NOESY of H23/27 shows weak enhancement of 12α, 12β, 16α, and 16β (Figure 2(d)). Comparison of the intensity of these enhancements

suggests a similarly short distance between H20 and 12α,β and between H20 and H14, as well as greater distances between H20 and 16α and between H23/27 and 12α, 12β, 16α, and 16β. Table 7 summarizes and compares the intensity of the observed NOE signals with expected NOE's based on H-H distances in all predicted low energy conformers of **1**. Comparison of these observed enhancements with expected NOE intensities for all predicted low energy conformers of **1** rules out conformers **1d** and **1e** as contributing conformers based on the absence of observable NOE signals involving H23/27 with H14 and 15α. The strong, equally enhanced NOE signals between H20 and 12α and between H20 and H14 suggest that the major conformer bears an extended side chain geometry, consistent with conformers **1b** and **1c**. In comparing conformers **1b** and **1c**, the weak NOE signal between H23/27 and 16α is consistent with the expected weak NOE intensity between H23/27 and 16α of conformer **1c** and inconsistent with the expected strong NOE intensity between H27 and 16α of conformer **1b**. Therefore, conformer **1c** is considered the major conformer.

The weak NOE signal between H23/27 and 12α,β, which is not expected to arise from conformers **1b** or **1c** since these conformers have distances greater than 5 Å between H23/27 and 12α,β, supports the presence of the syn orthogonal conformer **1a**.

In regard to conformations for **2**, the low frequency region of the 2D NOESY spectrum of **2** displays a strong cross peak between H20 and an overlapping region consisting of 12α, 12β, H14, and 15α. Additionally, weak cross peaks between H27 and 12α,β, 16α, and 16β are observable. This pattern of NOESY cross peaks is similar to that observed for **1**. An additional weak cross peak between H21 and 12α,β could also be detected. A selective 1D NOESY of H20 reveals that the strong cross peak consists

mainly of signal from H14 with some contribution from 12α,β (Figure 3(c)). The 1D NOESY of H20 also displays a very weak enhancement of 16α. The 1D NOESY of H27 displays the expected weak enhancements of 12α,β, 16α, and 16β expected from the 2D NOESY experiment (Figure 3(d)). The NOE data indicates the presence of at least two conformers with rotated phenyl rings since no predicted conformer of **2** is expected to have an NOE with H27 and both 12α and 16β.

As described in detail below, comparing these observed enhancements with expected NOE intensities for predicted conformers of **2** suggests that conformer **2c** is the major conformer with minor contribution from **2a** and other conformers as well (See Table 8).

The observed strong and moderately strong enhancements of H14 and 12α,β, respectively, upon irradiation of H20 suggests that the 17α side chain of the major conformer bears an extended geometry with a closer distance between H20 and H14 than between H20 and 12α,β. This is only consistent with conformers **2b** and **2c**, which have distances between H20 and H14 of 2.0 Å and 2.2 Å, and between H20 and 12α of 2.1 Å and 2.5 Å, respectively. Comparing **2b** and **2c**, the weak enhancement of 16α upon irradiation of H27 is consistent with the expected weak NOE intensity between H27 and 16α of conformer **2c**, but is inconsistent with the expected strong NOE intensity between H27 and 16α of conformer **2b**. Conformer **2c** thus is considered the major conformer.

As for minor conformers, conformer **2d** can be ruled out as a contributing conformer because of the absence of an observable NOE between H27 and H14 or 15α. For conformer **2f**, the expected weak enhancement of H14 upon irradiation of H20

suggests only a minor contribution since the observed enhancement is strong. The geometrically similar conformer, conformer **2e**, could not be ruled out with NOE data as a minor conformer. The presence of the syn orthogonal conformer **2a** is clear from the NOE enhancement of 12α,β upon irradiation of H27. All other conformers of **2** have a distance between H27 and 12α,β greater than 5 Å. The NOESY cross peak between H21 and 12α,β further supports the presence of conformer **2a** since all other predicted conformers bear a distance between H21 and 12α,β greater than 5 Å.

The low frequency region of the 2D NOESY spectrum of **3** displays additional cross peaks not found in the similarly patterned 2D NOESY of **1** and **2** (Figure 8). Aside from the cross peaks between H20 with 12α,β, H14, and 16α and H27 with 12α,β and 16α analogous to those observed for **1** and **2**, additional weak cross peaks between H21 and H27 with an overlapping region consisting of H14 and 15α appear. Also, weak cross peaks between the methylene protons of the 23-CH_2OH group and 12α,β and 16α are observable. A selective 1D NOESY of H20 reveals strong enhancements of H14 and 12α,β and weak enhancement of 16α (Figure 4(c)). The 1D NOESY of H27 displays the expected weak enhancements of 16α and the overlapped regions consisting of 12α,β and H14,15α (Figure 4(d)).

Comparing these observed enhancements with expected NOE intensities for predicted conformers of **3** indicates the presence of at least three conformers (see Table 9). The observed weak NOE enhancements of H21 with H14 and H27 with the overlapped region consisting of H14 and 15α are only consistent with the two predicted anti orthogonal conformers **3e** and **3f**. All other conformers of **3** have a distance between

these protons greater than 5 Å. Similarly, the observed weak NOE enhancements of H21 with 12α,β and H27 with 12α,β are only consistent with the two syn orthogonal conformers **3a** and **3c**. The very weak enhancement between the 23-CH_2OH metylene protons and 12α,β is only consistent with the predicted syn orthogonal/extended conformer **3g**.

As for the extended conformers, **3b** and **3d**, the strong NOE enhancements of 12α,β and H14 upon irradiation of H20 would be consistent with their presence. However, these strong NOE enhancements could reasonably result from an averaged contribution of the syn orthogonal conformers **3a** and **3c**, the anti orthogonal conformers **3e** and **3f**, and the syn orthogonal/extended conformer **3g**. The remaining extended conformer, **3h**, can not be ruled out with NOE data, but the expected strong enhancement of 16α,β upon irradiation of the methylene protons of the 23-CH_2OH group suggests only a minor contribution.

Discussion

The NOE data indicate that **1**, **2**, and **3** each exist in solution as an equilibrating mixture of conformers. Unlike **3**, both **1** and **2** show the dihedral C18-C17-C20-C21 restricted to a similar range of rotation. For **1** and **2**, the position of the 17α side chain ranged from the syn orthogonal conformers **1a** and **2a** to the anti orthogonal/extended conformers **1c** and **2e**, whereas for **3**, the 17α side chain ranged from the syn orthogonal conformers **3a**/ **3c** to the anti orthogonal conformers **3e**/ **3f**. In particular, the NOE data indicate that **1d** and **2d**, which are analogous to **3e**/ **3f** in side chain position, are not populated. Although the 17α side chain of **1** and **2** appears to have a similar range of

rotation, the NOE data do suggest that the relative populations of the major conformers of **1** and **2** are slightly different. For **1**, the NOE data indicates that the major conformer **1c** bears an anti orthogonal/extended 17α side chain, whereas for **2**, the major conformer **2c** has an extended 17α side chain. As for minor conformers, the NOE data suggests that the syn orthogonal conformer **2a** is more abundant in solution for **2** than **1a** is for **1.** This conclusion is rationalized from the H21, 12α,β cross peak found only in the 2D NOESY of **2**.

The presence of the anti orthogonal conformers only found in **3**, can be explained by stabilization experienced by **3e** and **3f** due to hydrogen bonding between the 17-OH and 23-CH_2OH groups. For **3**, intramolecular hydrogen bonding is not predicted for any of the other conformers according to the MM3 calculations.

The NOE data are mostly consistent with our statistical approach of evaluating contributing conformers from predicted ^{13}C shifts. The findings from multiple independent variable linear regression analysis of the ^{13}C data of **1** and **2**, that the major conformers **1c** and **2c** are 68% and 60% populated and that the minor conformers **1a** and **2a** are both 20% populated, are compatible with the identities of major and minor conformers favored by NOE data. Additionally for **3**, a 36% populated syn orthogonal conformer **3a**, 34% populated extended conformer **3d**, 28% populated anti orthogonal conformer, and 2% populated syn/extended conformer **3g** is quite consistent with the NOE data.

Consistent with the NOE data, the statistical analysis suggests that conformers **1b**, **1e**, and **2d** are not found in solution. For **1**, although a 12% contribution of conformer **1d** is inconsistent with the NOE data, perhaps this is only a minor inconsistency since the identity of the major conformer and another minor conformer are consistent in the two

methods. Furthermore, for **2**, a 20% population of conformer **2e** is consistent with the NOE data, although the NOE data do not clearly indicate that **2e** is the only additional minor conformer that is populated.

Conclusions

This study reveals that the substituent on the phenyl group of the 17α, Z-phenyl-vinyl substituent of estradiols can affect the conformational equilibrium of the 17α side chain. Hydrogen bonding stabilization between the 17-OH and a 23-CH_2OH substituent of **3** results in an additional anti orthogonal conformer not found in **1** or **2**. The similarity in solution conformations of 1 and 2 suggests they occupy a similar receptor volume that is consistent with their similar RBA of 20 and 23 at the estrogen receptor. The different conformational equilibria of **3** may explain its significant RBA of 140 which is greater than estradiol itself. Other effects such as hydrogen bonding, size, and electronic effects of the substituents may also play roles. These results can be applied to the design of subsequent ligands which will examine these conformational and substituent effects.

Experimental

NMR spectroscopy. ^{1}HNMR spectra were obtained on a Varian Unity INOVA instrument at 500 MHz. Chemical shifts were referenced to the residual ^{1}H signal of acetone-d_6, δ 2.04. COSY spectra were acquired with a spectral width of 4000 x 4000 Hz, accumulating 512 increments of 8 transients each with 0.15 s acquisition time and 1 s relaxation delay. Sine bell weighting and Gaussian apodization were used in both dimensions and the matrix was symmetrized after Fourier transformation. HMQC spectra were obtained for 5000 x 25000 Hz, accumulating 256 increments of 32 transients each with 0.2 s acquisition time

and 1.5 s relaxation delay. Gaussian apodization was used in both dimensions. NOESY spectra were acquired with a spectral width of 5000 x 5000 Hz, accumulating 256 increments of 32 transients each with 0.2 s acquisition time, 0.5 s mixing time, and 2 s relaxation delay. Gaussian apodization was used in both dimensions. 1D NOESY spectra were obtained with mixing times of 0.5 s using a spectral width of 3000 Hz. The relaxation delay and acquisition time were 2.0 s and 1.7 s, respectively, and up to 1000 scans were accumulated per spectrum. A Gaussian shaped pulse was used for selective irradiation. DEPT and ^{13}C spectra were obtained on a Varian Mercury instrument at 300 MHz.

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Figure Captions

Figure 1. Structures of **1**,**2**, and **3**.

Figure 2. (a) Low frequency spectral region of the 500 MHz ^{1}H NMR spectra of **1** in acetone-d_6. Equivalent spectral regions of the 500 MHz 1D NOESY spectra (500 ms mixing time) of **1** obtained by selective irradiation of the C18 methyl (b), H20 (c), and H23/27 (d) using a Gaussian pulse. Spectra (b) and (c) are 5x the vertical scale of (a). Spectra (d) is 10x the vertical scale of (a).

Figure 3. (a) Low frequency spectral region of the 500 MHz ^{1}H NMR spectra of **2** in acetone-d_6. Equivalent spectral regions of the 500 MHz 1D NOESY spectra (500 ms mixing time) of **2** obtained by selective irradiation of the C18 methyl (b), H20 (c), and H27 (d) using a Gaussian pulse. Spectra (b) and (c) are 5x the vertical scale of (a). Spectra (d) is 10x the vertical scale of (a).

Figure 4. (a) Low frequency spectral region of the 500 MHz ^{1}H NMR spectra of **3** in acetone-d_6. Equivalent spectral regions of the 500 MHz 1D NOESY spectra (500 ms mixing time) of **3** obtained by selective irradiation of the C18 methyl (b), H20 (c), and H23/27 (d) using a Gaussian pulse. Spectra (b) and (c) are 5x the vertical scale of (a). Spectra (d) is 15x the vertical scale of (a).

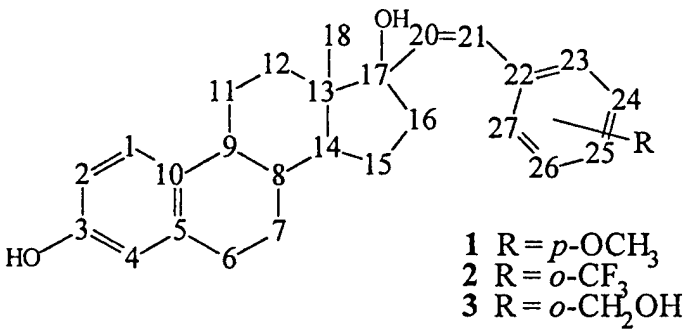
Figure 5. MM3-predicted geometries for the most stable conformers of **1**.

Figure 6. MM3-predicted geometries for the most stable conformers of **2**.

Figure 7. MM3-predicted geometries for the most stable conformers of **3**.

Figure 8. Spectral region of a 500 MHz 2D NOESY spectrum of **3** obtained with a mixing time of 500 ms. The NOE connectivities are indicated.

FIGURE 1



1 R = *p*-OCH_3
2 R = *o*-CF_3
3 R = *o*-CH_2OH

FIGURE 2

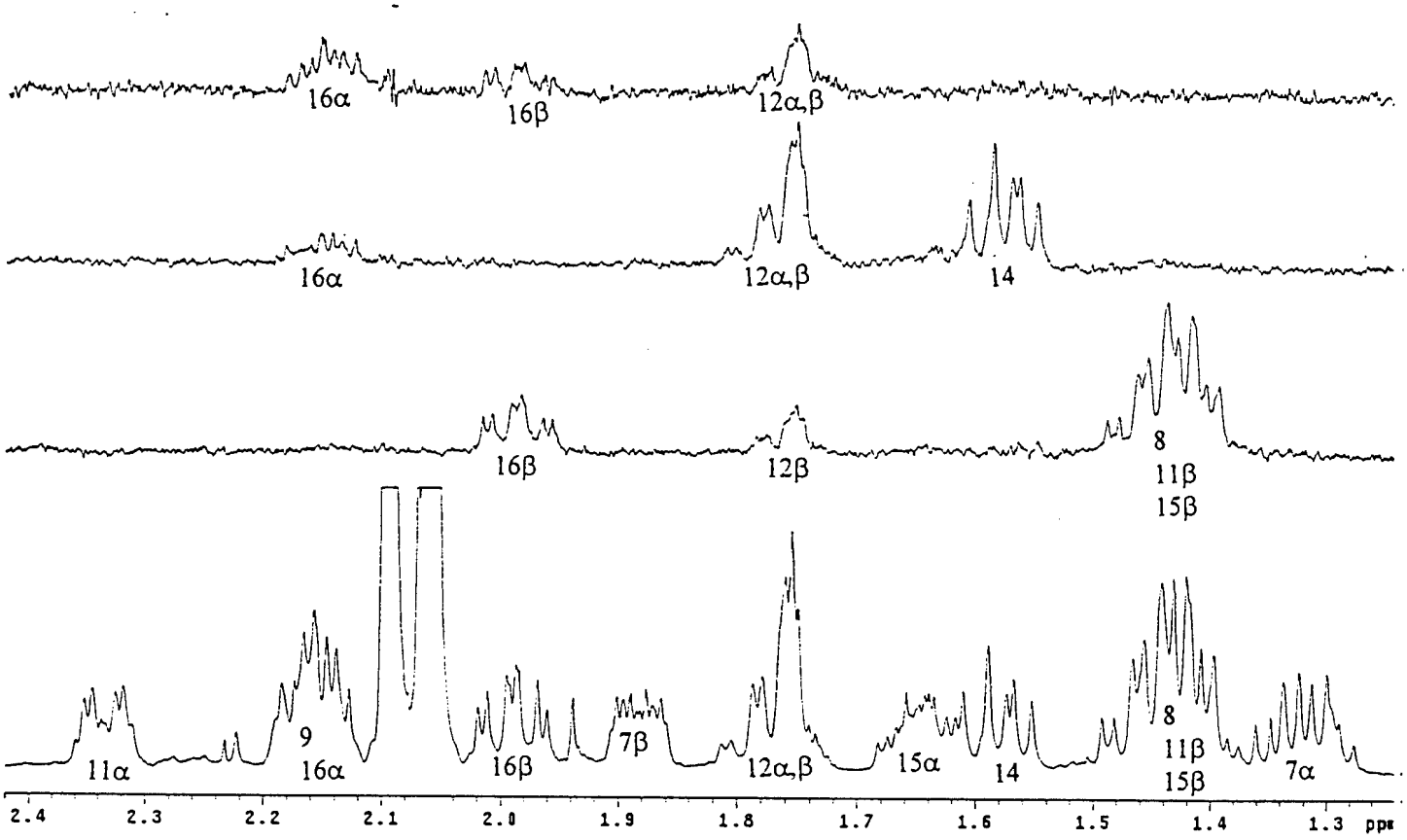

16α
16β
12α,β
16α
12α,β
14
16β
12β
8
11β
15β
11α
9
16α
16β
7β
12α,β
15α
14
8
11β
15β
7α
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
ppm

FIGURE 3

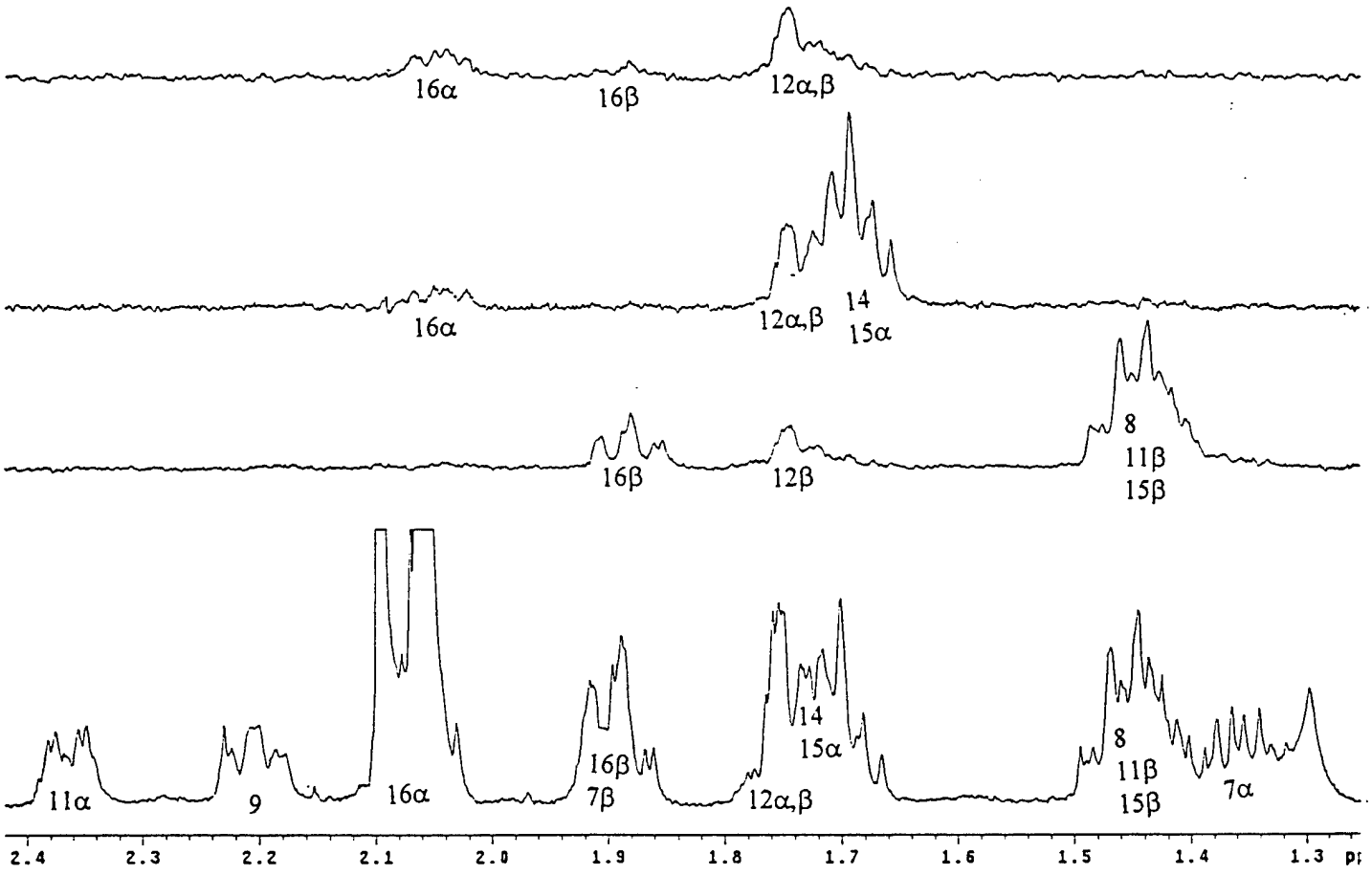
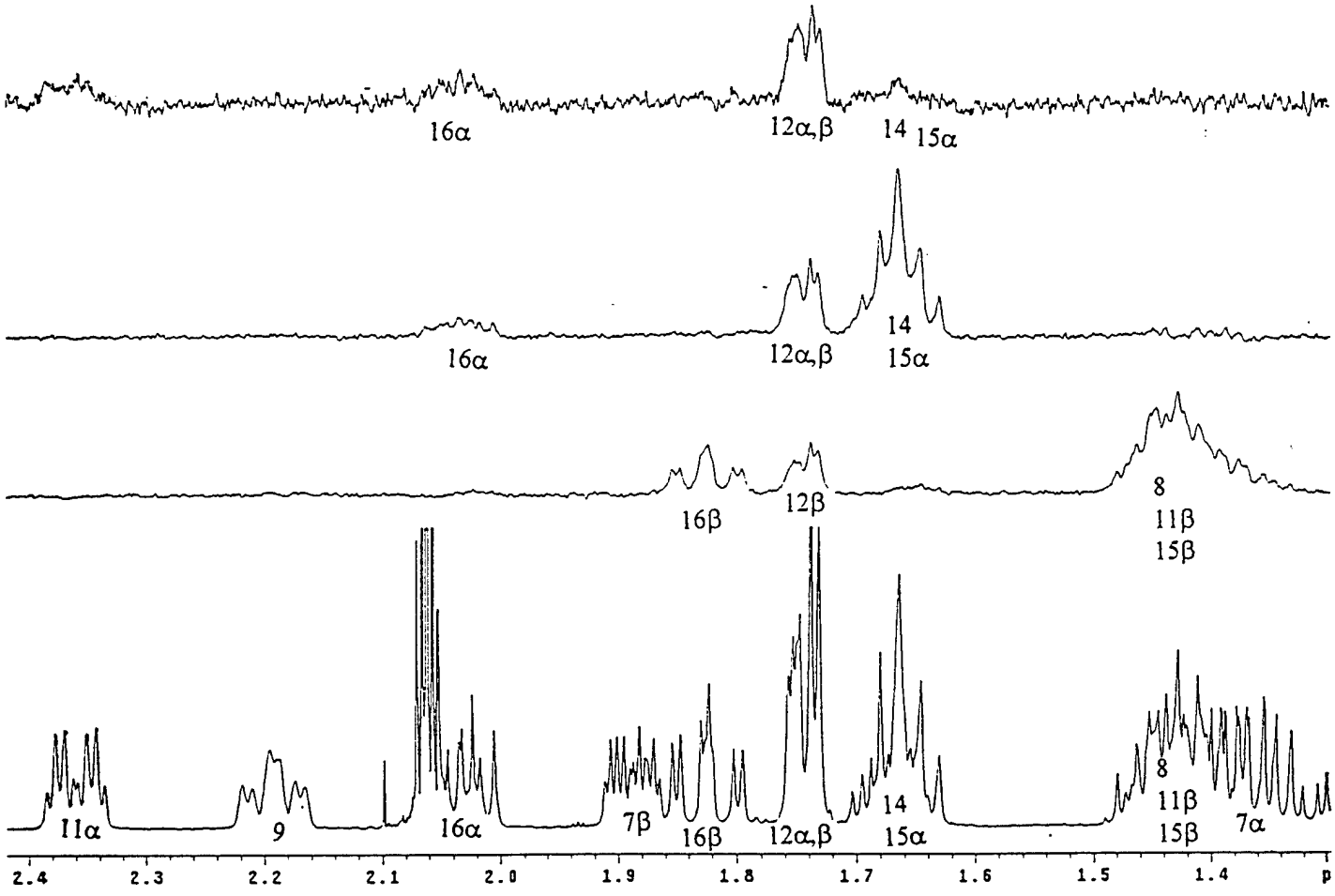

16α
16β
12α,β
16α
12α,β
14
15α
16β
12β
8
11β
15β
11α
9
16α
16β
7β
12α,β
14
15α
8
11β
15β
7α
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3

FIGURE 4


16α
12α,β
14
15α
16α
12α,β
14
15α
16β
12β
8
11β
15β
11α
9
16α
7β
16β
12α,β
14
15α
8
11β
15β
7α
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2.2
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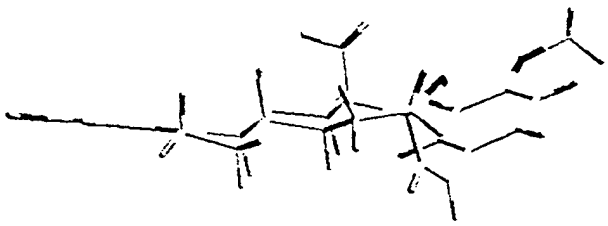
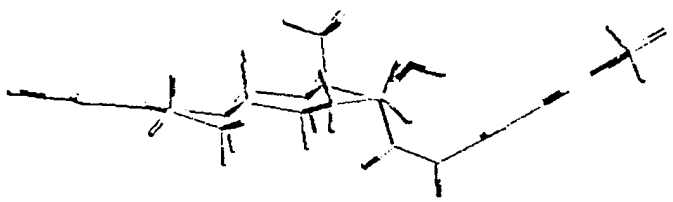
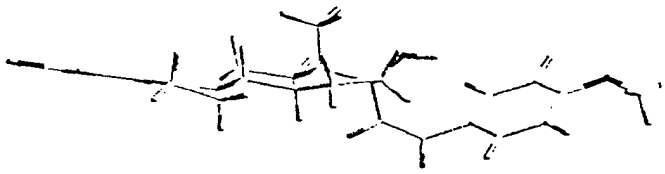
2.0
1.9
1.8
1.7
1.6
1.5
1.4
p



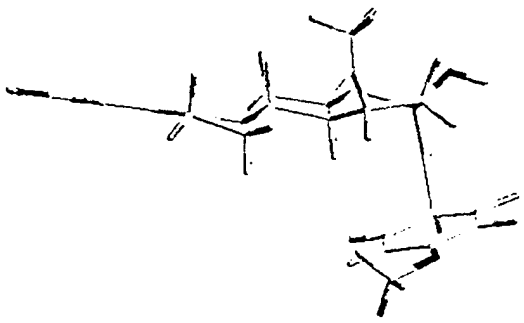
1a



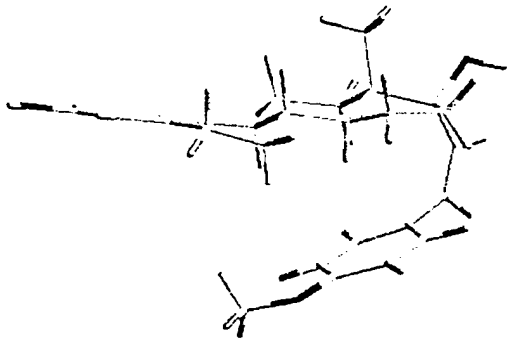
1b



1c

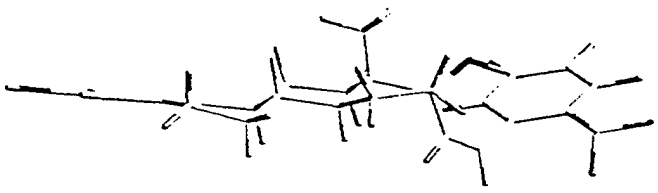


1d

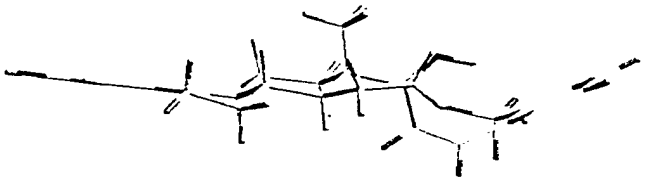


1e

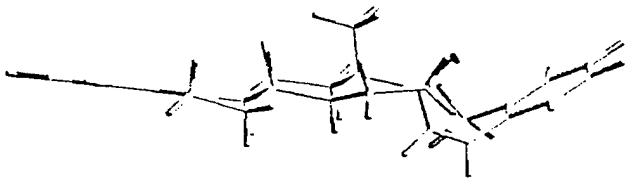
FIGURE 5



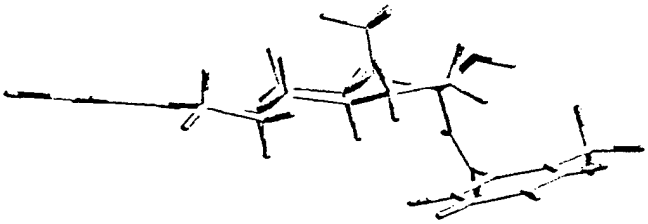
2a



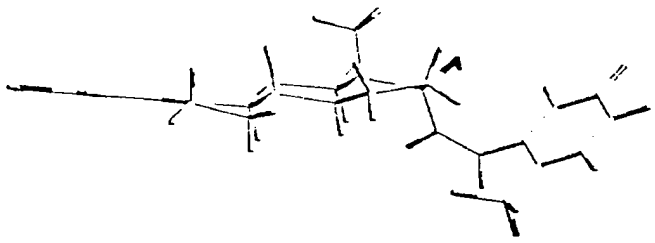
2b



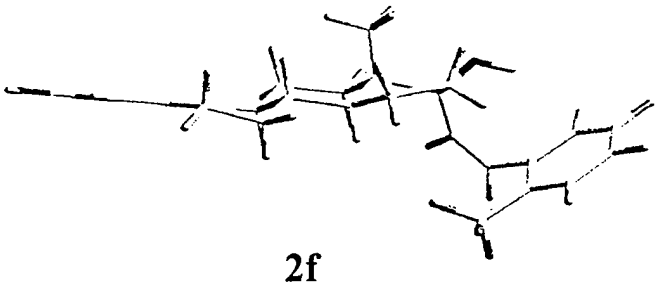
2c



2d

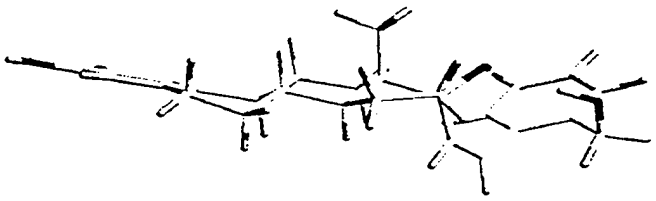


2e

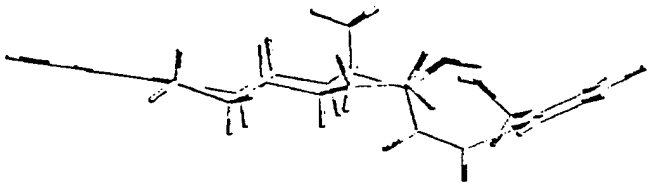


2f

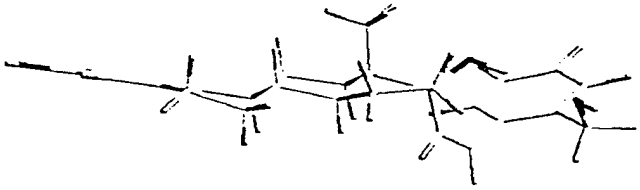
FIGURE 6



3a



3b



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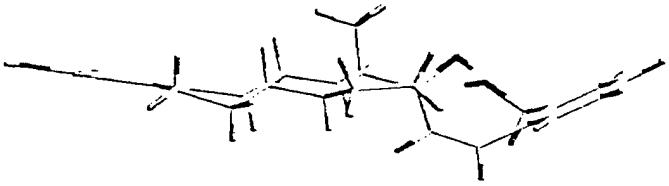
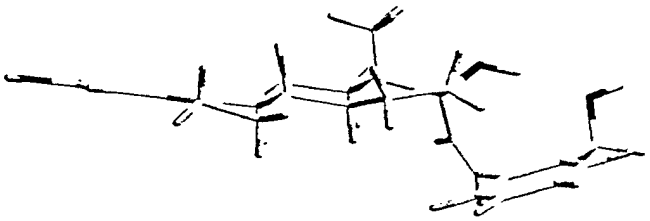
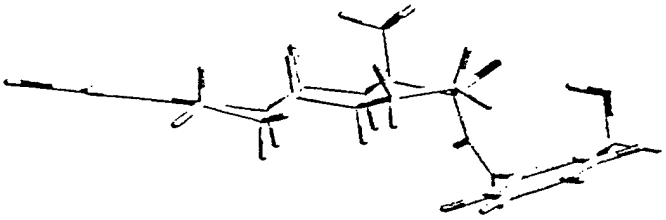


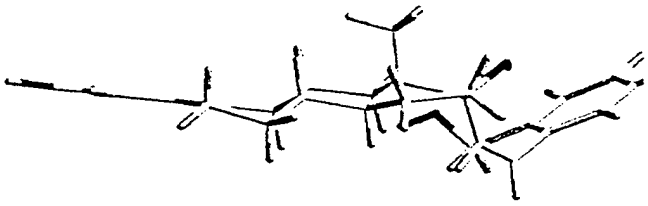
3d



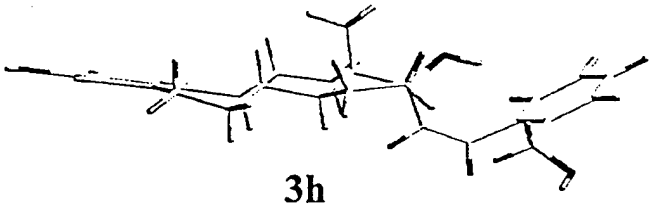
3e



3f



3g



3h

FIGURE 7

FIGURE 8

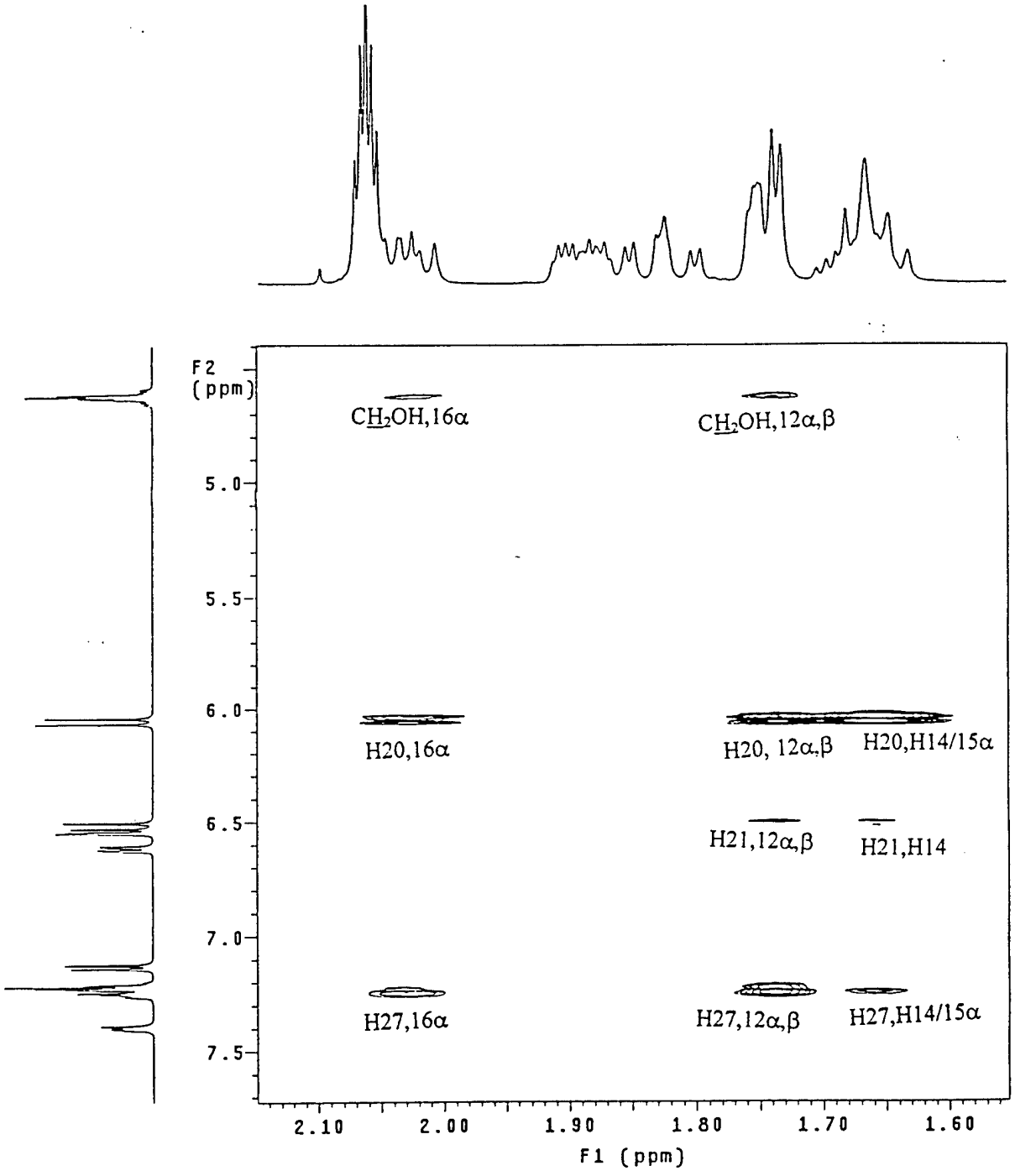

F2 (ppm)
5.0
5.5
6.0
6.5
7.0
7.5
CH2OH,16α
CH2OH,12α,β
H20,16α
H20, 12α,β
H20,H14/15α
H21,12α,β
H21,H14
H27,16α
H27,12α,β
H27,H14/15α
2.10
2.00
1.90
1.80
1.70
1.60
F1 (ppm)

Table 1. ^{1}H and ^{13}C Chemical Shifts for **1**, **2**, and **3**

^{1}H	1	2	3	^{13}C	1	2	3
1	7.12	7.12	7.12	1	126.9	127.4	126.5
2	6.60	6.62	6.62	2	113.5	113.9	113.2
4	6.54	6.60	6.58	3	155.8	155.0	153.2
6α	2.75	2.78	2.79	4	115.8	116.2	115.2
6β	2.80	2.81	2.82	5	138.3	139.1	137.5
7α	1.32	1.38	1.34	6	29.9	30.7	29.8
7β	1.88	1.88	1.88	7	28.5	28.7	27.9
8	1.43	1.48	1.43	8	40.7	41.2	40.2
9	2.18	2.20	2.18	9	44.5	45.0	44.0
11α	2.33	2.38	2.36	10	131.9	131.9	131.9
11β	1.46	1.45	1.46	11	27.3	27.7	26.8
12α	1.77	1.77	1.76	12	32.6	33.7	33.0
12β	1.75	1.75	1.74	13	48.7	49.0	48.0
14	1.57	1.70	1.66	14	49.9	50.8	49.9
15α	1.64	1.72	1.68	15	23.7	24.4	23.4
15β	1.41	1.43	1.40	16	38.3	39.3	38.4
16α	2.16	2.06	2.02	17	83.8	85.8	84.8
16β	1.98	1.90	1.82	18	14.5	14.8	14.6
CH_3	0.96	0.90	0.88	20	135.1	138.7	138.0
20	5.88	6.10	6.03	21	129.7	124.2	125.0
21	6.39	6.59	6.50	22	130.5	137.8	138.2
23	7.63	N/A	N/A	23	132.4	133.3	138.5
24	6.86	7.61	7.36	24	113.6	125.9	129.0
25	N/A	7.52	7.20	25	159.4	131.9	126.8
26	6.86	7.39	7.18	26	113.6	130.5	127.8
27	7.63	7.64	7.21	27	132.4	132.3	126.8
28[a]	3.80	N/A	4.60	28[a]	55.3	127.6	62.5

[a] Additional alkyl: 1, OCH_3; 2, CF_3; 3, CH_2OH

Table 2. Relative Energies and Key Dihedrals of Predicted Conformers of **1**, **2**, and **3** Using MM3

Conformers	C13-C17-C20-C21	C20-21-22-23	Relative Energies (kcal/mol)
1a	-103	-86	0
1b	-156	-68	0.6
1c	-110	-110	3
1d	105	86	3.2
1e	70	81	5.7
2a	-112	-99	0
2b	-151	109	0.3
2c	-148	93	1.7
2d	118	85	2.1
2e	162	-125	2.3
2f	145	-118	3.1
3a	106	90	0
3b	155	98	0.6
3c	109	94	2.3
3d	150	78	2.6
3e	-131	-81	3.3
3f	-132	-81	3.7
3g	111	105	4.2
3h	153	89	4.9

Table 3. Experimental and Predicted ^{13}C Chemical Shifts (ppm) of Predicted Conformers of **1** Using B3LYP/3-21G(X,6-31+G*)//MM3 Calculations

Carbon	1a	1b	1c	1d	1e	expt
C1	127.5	127.6	127.5	127.5	127.4	126.9
C2	113.0	113.0	113.1	113.0	112.8	113.5
C3	152.9	152.9	153.0	152.9	152.6	155.8
C4	115.6	115.6	115.7	115.6	115.7	115.8
C5	136.3	136.2	136.1	136.1	136.1	138.3
C6	31.0	31.1	31.1	31.0	30.7	29.9
C7	28.4	28.4	28.4	28.5	27.0	28.5
C8	40.1	39.8	39.7	39.6	38.7	40.7
C9	44.3	44.4	44.3	44.3	42.5	44.5
C10	132.1	132.1	132.2	132.3	132.3	131.9
C11	28.5	28.4	28.5	28.5	28.5	27.3
C12	34.2	32.0	31.9	32.8	34.0	32.6
C13	48.6	48.0	47.6	48.4	49.8	48.7
C14	50.7	48.7	49.1	49.0	47.3	49.9
C15	26.0	26.8	26.1	25.3	27.1	23.7
C16	39.8	46.6	38.1	37.5	46.6	38.3
C17	86.1	83.4	79.7	83.0	86.1	83.8
C18	16.0	15.3	14.3	15.1	16.1	14.5
C20	142.2	144.5	142.4	142.9	152.0	135.1
C21	133.1	130.8	134.8	135.3	134.5	129.7
C22	127.8	129.6	130.4	129.5	132.1	130.5
C23	129.2	130.1	127.4	131.7	129.1	132.4
C24	117.1	118.8	119.2	117.1	118.7	113.6
C25	157.5	157.5	157.6	156.9	157.4	159.4
C26	109.4	110.0	110.8	109.2	110.1	113.6
C27	132.2	128.6	128.8	129.6	130.9	132.4
C28	54.0	54.0	54.6	54.0	54.5	55.3

Table 4. Experimental and Predicted ^{13}C Chemical Shifts (ppm) of Predicted Conformers of **2** Using B3LYP/3-21G(X,6-31+G*)//MM3 Calculations

Carbon	2a	2b	2c	2d	2e	2f	expt
C1	127.3	127.6	127.6	127.5	127.6	127.4	127.4
C2	113.0	113.1	113.1	113.0	113.0	113.0	113.9
C3	153.0	153.0	152.9	153.0	152.9	153.2	155.0
C4	115.9	115.8	115.6	115.7	115.8	115.7	116.2
C5	136.0	136.0	135.9	136.1	136.3	136.3	139.1
C6	30.9	31.0	30.9	31.1	31.1	31.1	30.7
C7	28.3	28.4	28.4	28.4	28.1	28.0	28.7
C8	39.7	39.7	40.0	39.8	39.9	40.1	41.2
C9	44.1	44.0	44.4	44.1	43.9	44.0	45.0
C10	131.9	132.1	132.1	132.0	131.9	132.0	131.9
C11	28.5	28.4	28.3	28.6	28.4	28.6	27.7
C12	34.9	32.1	33.9	32.3	30.8	30.9	33.7
C13	48.0	47.9	49.3	48.0	47.7	48.0	49.0
C14	50.1	49.5	50.5	49.1	49.1	48.7	50.8
C15	26.5	26.8	26.3	26.3	25.8	26.1	24.4
C16	42.6	45.1	39.3	35.0	40.6	36.1	39.3
C17	86.1	84.4	87.8	81.8	81.8	80.5	85.8
C18	14.6	15.1	16.1	14.7	15.4	15.0	14.8
C20	143.7	147.0	141.6	142.0	145.5	140.6	138.7
C21	127.8	126.2	129.2	129.4	132.0	133.3	124.2
C22	138.9	138.9	135.5	139.8	139.2	140.5	137.8
C23	129.2	133.4	131.5	132.4	131.1	130.5	133.3
C24	125.9	127.2	127.4	126.6	129.2	128.1	125.9
C25	130.7	128.8	131.1	130.6	130.8	130.4	131.9
C26	131.7	131.0	130.2	131.4	132.0	131.7	130.5
C27	132.2	128.8	134.5	129.4	129.6	130.8	132.3
C28	127.0	127.1	127.4	126.7	127.3	127.0	127.6

Table 5. Experimental and Predicted ^{13}C Chemical Shifts (ppm) of Predicted Conformers of **3** Using B3LYP/3-21G(X,6-31+G*)//MM3 Calculations

Carbon	3a	3b	3c	3d	3e	3f	3g	3h	expt
C1	127.4	127.6	127.4	127.4	127.5	127.3	127.3	127.3	126.5
C2	113.1	113.2	112.9	112.9	112.8	113.0	112.9	113.0	113.2
C3	153.1	153.0	152.8	152.9	152.8	153.1	152.9	152.9	153.2
C4	115.9	115.7	115.7	115.7	115.6	115.8	115.7	115.8	115.2
C5	136.0	136.0	136.1	136.3	136.3	136.3	136.1	136.1	137.5
C6	30.9	31.0	31.0	31.1	31.2	31.0	31.1	31.0	29.8
C7	28.2	28.5	28.4	28.5	28.3	28.1	28.5	28.4	27.9
C8	39.8	39.7	39.6	40.0	40.0	40.1	40.1	39.6	40.2
C9	44.0	44.1	44.2	44.4	44.0	43.8	44.5	44.3	44.0
C10	131.7	131.8	132.5	132.3	132.4	131.4	132.2	132.1	131.9
C11	28.4	28.3	28.6	28.5	28.7	28.4	28.6	28.5	26.8
C12	34.3	31.3	34.9	31.9	31.7	30.4	33.0	32.3	33.0
C13	48.0	47.5	47.8	48.0	47.7	48.1	49.1	48.3	48.0
C14	50.4	49.2	50.1	49.2	49.3	49.1	50.8	49.3	49.9
C15	26.2	26.6	26.8	26.1	25.7	25.0	26.2	26.7	23.4
C16	39.4	44.9	43.3	42.0	34.4	30.9	39.2	43.6	38.4
C17	85.9	83.3	85.4	84.4	79.9	80.2	87.3	85.8	84.8
C18	16.1	15.3	14.7	16.0	15.5	15.4	16.0	14.9	14.6
C20	141.6	144.6	145.0	146.1	142.8	136.2	141.7	141.5	138.0
C21	130.9	129.6	128.3	127.8	129.2	136.5	130.5	127.8	125.0
C22	134.5	136.4	140.2	140.6	140.5	135.5	133.4	134.3	138.2
C23	141.0	140.6	133.2	133.5	135.9	140.4	136.4	140.0	138.5
C24	131.9	131.7	131.4	131.2	132.5	131.8	130.0	130.4	129.0
C25	128.5	126.8	128.5	126.9	126.9	127.9	127.7	127.9	126.8
C26	126.0	128.3	127.0	128.8	128.3	126.2	126.2	126.6	127.8
C27	131.9	131.7	131.4	131.2	127.7	131.8	132.8	126.5	126.8
C28	64.5	65.0	65.9	66.2	64.7	64.1	63.2	63.3	62.5

Table 6. Summary of the Multiple Independent Variable Regression Analysis[a] of the Calculated ^{13}C Shifts of Predicted Conformers of **1**, **2**, and **3**

Conformer	Estimate (%)	Standard Error (%)
1a	20	12
1b	0	7
1c	68	24
1d	12	30
1e	0	0
2a	20	13
2b	0	15
2c	60	1
2d	0	7
2e	0	11
2f	20	8
3a	36	14
3b	0	1
3c	0	5
3d	34	26
3e	28	14
3f	0	1
3g	2	7
3h	0	10

[a] Constraints: Each conformer is greater than or equal to 0 %. Conformer sets **1a-1e**, **2a-2f**, and **3a-3h** total to 100 % each.

Table 7. Summary and Comparison of Observed NOE Enhancements with Expected NOE Intensities[a] for Predicted Conformers of **1**

Irradiated	Enhanced	1a	1b	1c	1d	1e	Expt
H20	12α,β	w	s	s	s	w	**s**
H20	H14	s	s	s	w	w	**s**
H20	16α	s	w	w	w	w	**w**
H23/27	12α,β	s	n	n	w	s	**w**
H23/27	H14	n	n	n	s	s	**n**
H23/27	15α	n	n	n	w	s	**n**
H23/27	16α	n	s	w	s	s	**w**
H23/27	16β	n	w	w	w	s	**w**

[a] Expectations of strong (s), weak (w), and no (n) NOE enhancements correspond to H-H distances of 0 - 2.99; 3.0 - 4.99; and > 5 Å.

Table 8. Summary and Comparison of Observed NOE Enhancements with Expected NOE Intensities[a] for Predicted Conformers of **2**

Irradiated	Enhanced	2a	2b	2c	2d	2e	2f	Expt
H20	12α,β	w	s	s	s	s	s	**s**
H20	H14	s	s	s	w	s	w	**s**
H20	16α	s	w	w	w	w	w	**w**
H21	12α,β	w	n	n	n	n	n	**w**
H27	12α,β	s	n	n	w	n	n	**w**
H27	H14	n	n	n	s	n	n	**n**
H27	15α	n	n	n	s	n	n	**n**
H27	16α	n	s	w	w	n	s	**w**
H27	16β	n	w	w	n	w	w	**w**

[a] Expectations of strong (s), weak (w), and no (n) NOE enhancements correspond to H-H distances of 0 - 2.99; 3.0 - 4.99; and > 5 Å.

Table 9. Summary and Comparison of Observed NOE Enhancements with Expected NOE Intensities[a] for Predicted Conformers of **3**

Irradiated	Enhanced	3a	3b	3c	3d	3e	3f	3g	3h	Expt
H20	12α,β	w	s	w	s	s	s	s	s	s
H20	H14	s	s	s	s	w	w	s	s	s
H20	16α	s	w	s	w	w	w	s	w	w
H21	12α,β	w	n	w	n	n	n	n	n	w
H21	H14	n	n	n	n	w	w	n	n	w
H27	12α,β	s	n	s	n	n	n	n	n	w
H27	H14	n	n	n	n	w	w	n	n	w
H27	15α	n	n	n	n	w	w	n	n	w
H27	16α	n	s	n	s	w	w	w	n	w
CH_2OH	12α,β	n	n	n	n	n	n	s	n	w
CH_2OH	16α	w	n	w	n	w	w	n	s	w

[a] Expectations of strong (s), weak (w), and no (n) NOE enhancements correspond to H-H distances of 0 - 2.99; 3.0 - 4.99; and > 5 Å.

Synthesis of Auger Electron-Emitting Radiopharmaceuticals

Robert N. Hanson*

Department of Chemistry, Northeastern University, Boston, MA 02115 USA

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Abstract: Targeted radiotherapy using Auger electron-emitting pharmaceuticals offers both advantages and challenges compared to alternative α - or β -emitting agents. The low energy Auger electrons deposit their energy within the target cell thereby minimizing collateral damage. To achieve this effect, however, the radiopharmaceutical must incorporate the appropriate radionuclide, be efficiently synthesized, and once administered, be distributed selectively to its biological target. This review covers the synthesis of agents which have prepared over the past decade either as Auger electron-emitting radiopharmaceuticals or which have the potential as such. While not an exhaustive review, the major classes of agents, such as hormone receptor ligands, nucleoside analogs and intercalating agents are described.

I.INTRODUCTION

Targeted radiotherapy, using internally emitted radiation, offers an alternative to the use of traditional radiation therapy or boron neutron capture therapy. The key features in this modality include the ability to direct the agent to the target tissue using a biological marker, the deposition of high linear energy transfer (LET) radiation at the site in a short period of time, and to have that energy transfer result in a localized cytotoxic event. The result of this process is to cause a high lethality rate among targeted populations of cells, often neoplastic cells, while generally sparing neighboring normal or nontargeted cells. Aspects of this process, e.g., use of antibodies and oligonucleotides to target cells, microdosmetry and the use of alpha-emitting radionuclides, are discussed in accompanying reviews in this issue.

Unlike β- or α-emitting radionuclides, which deposit their LET effects over several cell diameters, the low energy Auger electrons emitted during radioactive decay deposit their energy within subcellular dimensions [1-3]. As a result, for a compound labeled with an Auger electron-emitting radionuclide to exert a cytotoxic effect, it has to be able to penetrate within the cell. In addition, for the agent to generate a lethal event, that localization should be within the proximity of the nuclear DNA. As described elsewhere, and previously reported, cell death is associated most closely with the ability to cause double strand breaks in the DNA as a consequence of the shower of low energy electrons. Therefore, for an Auger electron-emitting radiopharmaceutical to have therapeutic potential, 1. a radionuclide must have an appropriate radiation decay profile, 2. a radionuclide should be able to be economically prepared in reasonably high specific activity and purity, 3. a radionuclide should be incorporated efficiently into a carrier molecule, 4. a carrier molecule should display biodistributional selectivity for the target tissue, and 5. in the target tissue, the agent should associate with the nuclear DNA complex for a time consistent with the half-life of the radionuclide. To date, virtually no Auger electron-emitting radiopharmaceutical has met all of these criteria. However, sufficient data both from *in vitro* studies with putative Auger emitters and from α/β/γ-emitting radiopharmaceuticals suggest that success may be achieved with improved targeting mechanisms.

Based on the previously listed criteria, one is left with a relatively small set of available radionuclides with which to work (Table 1).

Table 1. Auger Electron-Emitting Radionuclides for Use in Radiopharmaceutical Synthesis

Chromium-51	Gallium-67	Bromine-77, 80m
Indium-111	Iodine-123, 125	Platinum-193m
Thallium-201		

*Address correspondence to this author at the Department of Chemistry, Northeastern University, Boston, MA 02115 USA; Phone +1-617-373-3313; Fax: +1-617-373-8795; e-mail: ro.hanson@nunet.neu.edu

1381-6128/00 $19.00+.00

The most prominent of the Auger emitting radionuclides are the isotopes of iodine (I-125 and I-123) and bromine (Br-77 and 80 m). To a much less degree, studies have been reported related to the Auger effects of In-111 and Pt-193m. The other radionuclides that emit Auger electrons as part of their decay scheme, however, either have other emissions (γ, β+, β-), half-life considerations or production characteristics that preclude their use as potential Auger-radiotherapeutics. The chemical properties of the radiohalogens allow them to be more readily incorporated into organic molecules by traditional synthetic methods, whereas the metal ions require chelation techniques [4,5]. These two strategies, as shown later, influence the types of targeting agents to which they are bound.

The low energy of the Auger electrons requires that they be emitted as close to the nucleus of the cell as possible to exert their lethal effect. Therefore, the carrier molecule for the radionuclide has to cross the cell membrane either by passive diffusion or via a specific carrier mediated process. Once inside the cell, the carrier-radionuclide complex has to bind selectively to the DNA or a DNA associated protein. This criterion dramatically reduces the number of potential carriers available for molecular manipulations (Table 2).

Table 2. Mechanisms for Nuclear/Intracellular Localization

1.	Nuclear Receptor Binding
2.	DNA-directed Agents
3.	Other Intracellular Targets

For the treatment of cancers with Auger emission radiotherapy, the most promising carrier molecules are the steroid hormones (via their receptors), DNA directed agents (nucleosides, intercalators, groove binding) and a few proteins and peptides [6]. Given the available radionuclides, there are relatively few options to exploit. This is in a distinct contrast to those β- or α-emitting agents which do not require that degree of localization.

The primary objective of this review is to cover the progress since 1990 [7] in the preparation of radiotherapeutic agents bearing (potential) Auger electron-emitting radionuclides. Because the biophysical constraints imposed on this approach have limited its utility, a secondary objective will be to consider potential agents, based on work done with other radiodiagnostic or radiotherapeutic materials.

II. HORMONE RECEPTOR LIGANDS

The mechanism of action of the steroid hormones has made the preparation of labeled analogs one of the major foci of radiopharmaceutical development. Receptors for the endogenous hormones are overexpressed in a number of human carcinoma cell lines. The circulatory steroids enter all cells by passive diffusion, however, only responsive cells contain the requisite hormone receptor. Binding of the hormone to its cognate receptor in the nucleus of the cell initiates a series of events which includes the binding of the steroid-receptor complexes to the nuclear DNA. The high affinity for the receptor, the selectivity of the hormone-receptor interactions, and the avidity of the complex for the DNA combine to provide the basis for radiotherapy using Auger electron-emitting steroid hormone receptor ligands [8]. Although success in achieving the affinity and selectivity for the estrogen receptor has been the greatest, synthesis of radiolabeled androgen and progestin receptor ligands have been reported in the past 10 years.

A. Estrogen Receptor Ligands

During the 1980's the synthesis of a number of radiohalogenated analogs of estradiol were reported. The reviews by Katzenellenbogen [9] and Cummins [10] describe the labeling methods and biological properties of many of these ligands. While most of the emphasis was focused on the radiodiagnostic potential of these agents, the presence of Auger electrons from the decay of I-123/125 and Br-77/80 m initiated interest in their radiotherapeutic applications. The compounds that were most extensively evaluated were the 16α-halogenated (I/Br)-estradiols and the 17α-halo (I/Br) vinyl estradiols. The former were prepared by nucleophilic displacement of the appropriately substituted 16β- X-estradiol. The latter were synthesized using the radiohalodestannylation methodology that we developed in the early 1980's. Both methods provided target compounds rapidly and in high yields (Fig. **1**). Studies with these agents demonstrated that the presence of the halogen at either positions was tolerated or, in the case of the 17α-halovinyl estrogens, beneficial to binding. Additional substituents at the 11β or 7α positions also enhanced receptor binding. *In vitro* studies indicated that radiocytotoxicity was receptor mediated and, therefore, validated this approach.

More recent synthetic approaches have focused on two aspects, the enhancement of affinity within the estradiol structure, or identification of

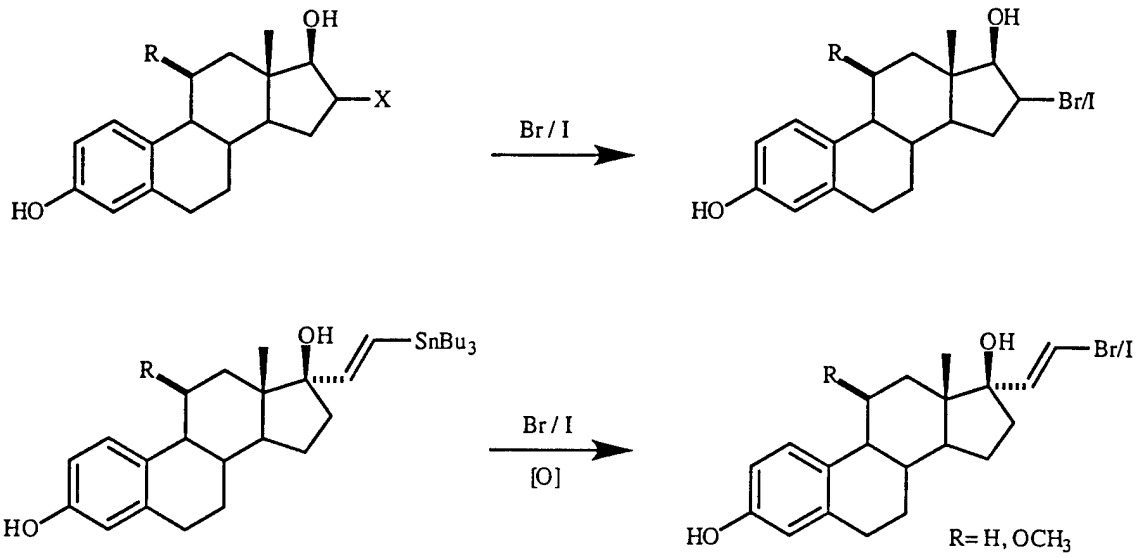


Fig. (1). Radiobromination/iodination of estradiols.

nonsteroidal estrogens with possibly better pharmacokinetic properties. Because both approaches utilized the destannylation methodology for introduction of the Auger emitting radiohalides, the challenges were primarily associated with the synthesis of the precursor trialkylvinylstannanes. Previous studies [11] had demonstrated that the 17α-Z-halovinyl estradiols had higher affinity than the corresponding 17α-E-isomers. Small lipophilic substituents at the 11β-position provided an additional enhancement of relative binding affinity (RBA) [12]. The synthesis of the 11β-vinyl/ethyl 17α-Z-tributylstannylvinyl estradiol precursor for radiohalogen labeling is shown in Fig. **2**. The process involved at least 13 steps with an overall yield of <2%, prior to the radiohalogenation (the E-isomer can be obtained in ~4% overall yield). As a result, few of these analogs have been evaluated *in vitro* or *in vivo*. Initial data suggest that the radiocytotoxicity is retained, however, the physicochemical properties of the individual compounds produce variations in the pharmacokinetics. Additional work by Cummins [13] and Quincy [14] have also utilized the 17α -iodovinyl group to prepare labeled estrogenic ligands, although with imaging as the objective.

The alternate approach for estrogen receptor ligands utilizes a nonsteroidal structure. DeSombre, *et al.* prepared the [Br-80m] labeled bis(hydroxyphenyl)ethylene [15]. While initially prepared via direct radiobromination of the

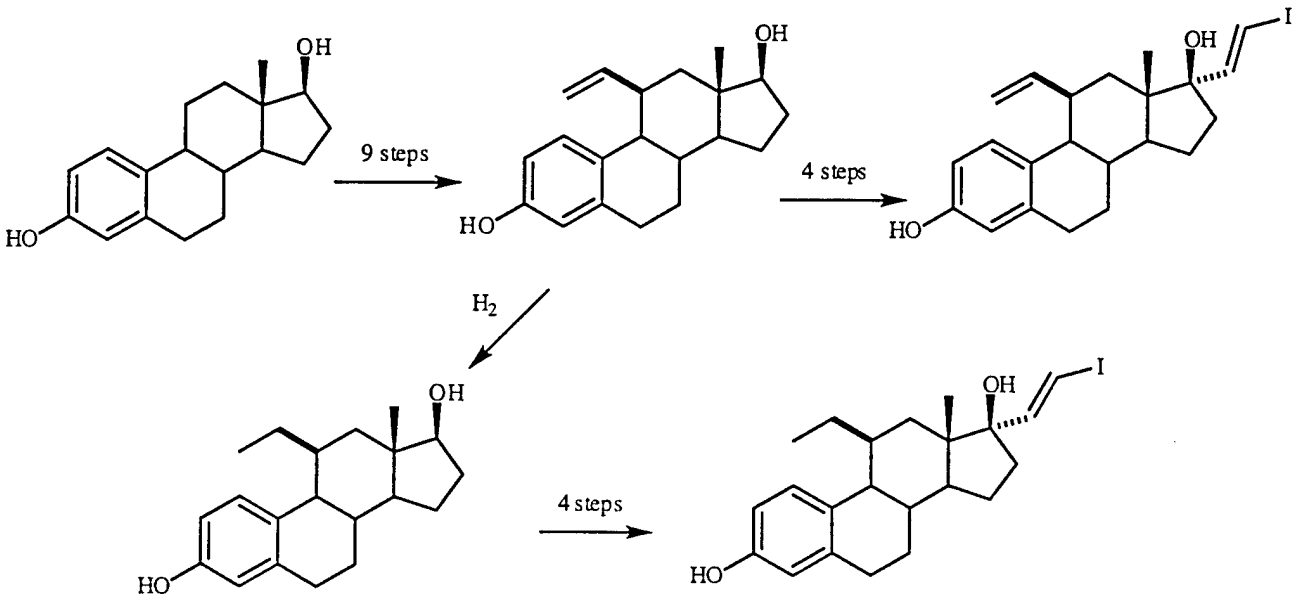


Fig. (2). Synthesis of 11β-substituted estradiols.

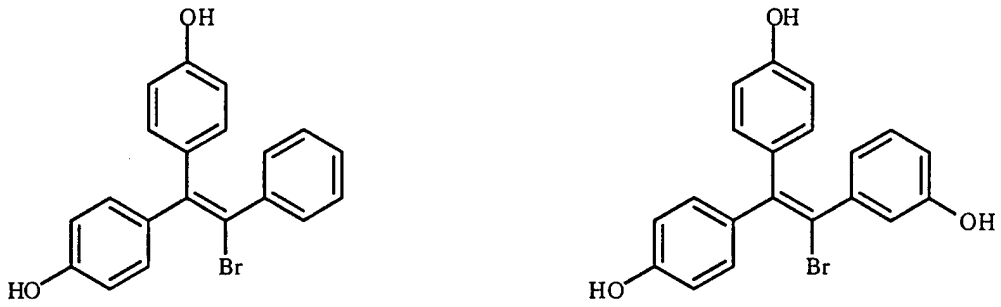


Fig. (3). Bis- and tris-hydroxy-triphenylethylene bromide.

protected material, better yields of purer product were obtained by using the destannylation methodology. Comparison with the 11β-substituted 17α-iodovinyl estradiols suggested that some pharmacokinetic advantages were associated with the nonsteroidal structure. In order to improve receptor binding, an analog with an additional phenolic group has been prepared (Fig. **3**). The initial synthesis of the stannyl intermediate was achieved using transmetallation of the vinyl bromide with alkyl lithium followed by quenching with trialkyltin halide, however, the yield in the final step was low. Use of hexabutylditin and Pd(0)catalyst raised the yield by an order magnitude. Biological studies with these labeled products (Br-80m/I-123) are currently undergoing *in vitro* evaluation.

An alternate approach to the use of labeled estrogenic agonists is the preparation of antagonists. Although both steroidal and nonsteroidal antagonists have been described in the literature, only labeled derivatives of nonsteroidal antagonists have been reported. For example, iodoxifene has been prepared and evaluated as a selective estrogen receptor modulator (SERM) and its resynthesis with the addition step for replacement of iodine by tributyltin would provide the immediate precursor for labeling with either of the isotopes of iodine (Fig. **4**).

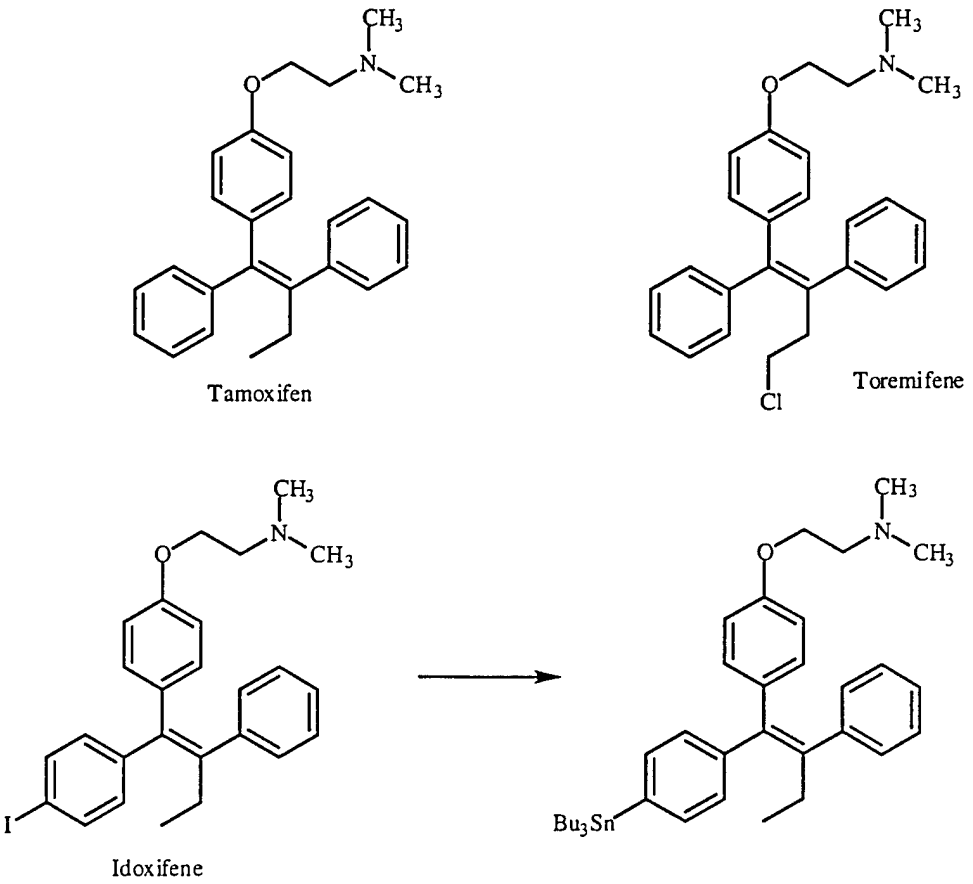


Fig. (4). Nonsteroidal estrogen receptor ligands (antiestrogens).

B. Progesterone Receptor Ligands

The design of radiolabeled progesterone receptor seeking ligands, as described by Brandes and Katzenellenbogen, has been hampered by several factors [16,17]. A major problem is that the endogenous ligand, progesterone, has a binding affinity for its receptor that is almost an order of magnitude less than that of estradiol for the estrogen receptor, 4.5 x 10^{-9}M vs. 3 x 10^{-10}M. As a consequence, a ligand receptor complex is less likely to remain associated with the nuclear DNA long enough for therapeutically relevant Auger emitting radionuclides to deposit their energy at the site. In addition, structure-activity studies on the progesterone receptor ligands provided relatively few examples of compounds that had relative binding affinities (RBA) significantly greater than progesterone itself. Among that subset, even fewer were amenable to radiolabeling at sites that would be chemically or metabolically stable (Fig. **5**). During the 1980's Hochberg, *et al.* described the preparation of the 17α-iodovinyl testosterone (ethisterone) and 19-nortestosterone (norethisterone) analogs in their radiolabeled form using the halodestannylation methodology [18,19]. The Schering group also explored these as potential ligands for the progesterone receptor [20].Salman, *et al.* introduced the radiohalogen at the terminus of a 17α-haloalk-1-ynyl-19-nortestosterone in an attempt to enhance the affinity of the compound for the receptor [21]. While these compounds were chemically stable and relatively resistant to metabolism, they displayed little ability to localize in progesterone receptor rich tissue, to be retained there or exert any radiocytotoxic effect.

Since 1990, most of the efforts in the area have focused on the radiodiagnostic applications of the labeled progestins [22]. A number of the syntheses, however, employed labels that could be considered for radiotherapy given the appropriate radionuclide. Examples of these syntheses are shown in Fig. **6** and the putative radiosynthesis with the Auger emitting nuclide is provided. Van Lier's group synthesized the 17α -iodovinyl testosterone and 19-nortetstosterone derivatives and evaluated their radioiodinated forms as ligands for the progesterone (and androgen) receptors [23]. Their results essentially confirmed previous findings regarding the inadequacy of the ligands.

Based on the studies of Brandes and Katzenellenbogen which were primarily directed to F-18-labeled progesterone ligands, Van der Bos and Rijks prepared and evaluated a series of four iodinated progestins [24,25]. Two were the E- and Z-isomers of 17α-iodo-19-nortestosterone previously evaluated, two were the E- and Z-isomers 17β-hydroxy-17α-iodovinyl-11-methylene-19-norgon-4-ene-3-one [ORG 3236 analogs), and the 21-iodophenoxy-16-α-ethyl-19-norpreg-4-ene-3,20-dione (ORG-2058 analog). The two ORG 3236 compounds had RBA values

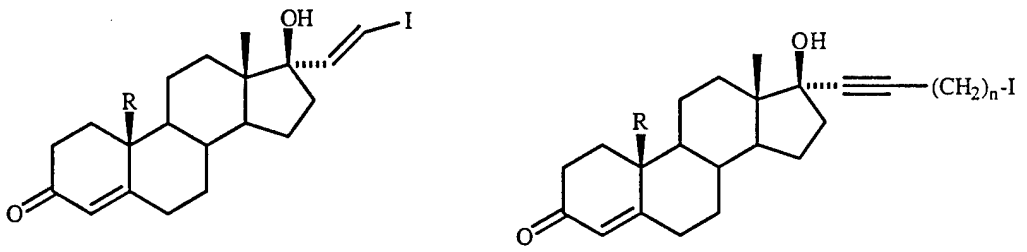
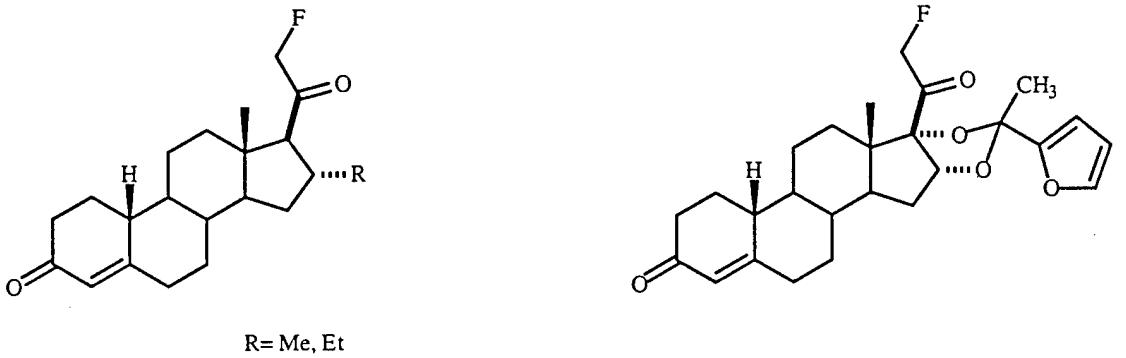


Fig. (5). Radiolabeled Derivatives of ethisterone and norethisterone.



R= Me, Et

Fig. (6). Radiofluorinated progesterone receptor ligands.

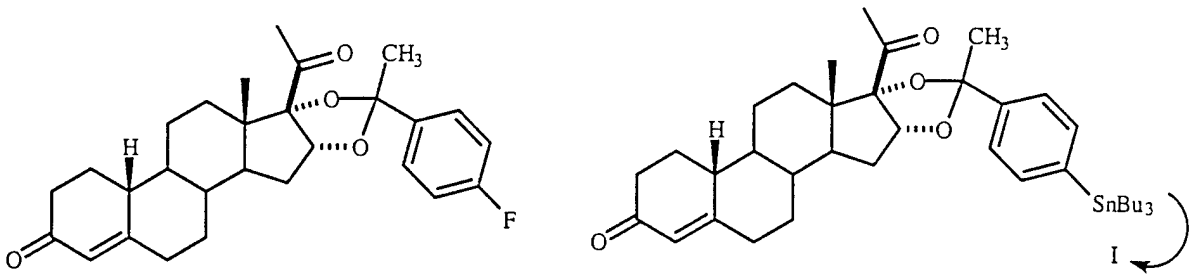


Fig. (7). Use of tributylstannyl analog as precursor for iodine radionuclide.

specifically greater than progesterone while the ORG 2058 analog bound with only 7% of the affinity of the endogenous ligand. Radiolabeling was achieved via the corresponding tributylstannyl precursor in good yields and high radiochemical purity. *In vivo* tissue distribution studies were disappointing for all of the ligands. Only the Z-isomer of the iodovinyl ORG-3236 analog possessed selectivity for the progesterone rich tissues in normal female rat. However, this selectivity was not observed in the induced mammary tumors.

Although the studies focused on imaging, the failure to be retained by the target tissues would also be of concern for radiotherapeutic applications as well.

Reevaluation of the work of Katzenellenbogen may provide additional possibilities for radioiodinated analogs of progesterone receptor ligands (Fig. **7**). In particular the work with the 16α, 17α-dioxolanes provides opportunities to synthesize the corresponding iodinated analogs of the fluorinated compounds [26-28]. Conversion to the corresponding tributylstannyl derivatives followed by radioiododestannylation should yield target radiochemicals for *in vitro* and *in vivo* evaluation. Whether such products would overcome the deficiencies seen with previous agents, i.e., reduced affinity high nonspecific binding or metabolic lability, remains to be seen. A novel variation which would be amenable to the incorporation of a Auger-emitting metal ion has also been reported by this group [29].

C. Androgen Receptor Ligands

Many of the same limitations imposed on progesterone receptor-directed ligands are encountered in the chemistry of the androgen receptor targeted agents. The endogenous ligands, testosterone and 5α-dihydrotestosterone have receptor affinities an order of magnitude less than that observed for estradiol at the target site. While there is an extensive literature related to androgenic and anabolic steroids, few of those compounds have higher affinities than 5α-DHT for the target receptor. In addition, the endogenous ligands are rapidly metabolized to products with much lower receptor affinities. As a result, very few compounds have been described which have high affinity, metabolic stability and the potential for incorporation of a radionuclide possessing the desired properties.

The work with radiolabeled androgenic steroids over the past 10 years has concentrated primarily on their radiodiagnostic (PET and SPECT) potential. This mostly represented an extension of studies conducted during the late 1980's in which radiohalogens I-125 or F-18 were incorporated at the 7α, 16α, or 17α-positions (Fig. **8**) [30-33]. These early results were generally disappointing in that the radiochemicals exhibited either little specific binding or metabolic lability, or both. The challenges, therefore, were to improve the receptor affinity and the stability of the C-I bond.

Hochberg and co-workers extended their studies of the 17α-[^{125}I]-iodovinyl testosterone and nortestosterone radioligands with the preparation of E- and Z-17α-iodovinyl-7α-methyl nortestosterone. The E-isomer was twice as potent as the Z-isomer but still less than 5α-DHT (RBA = 12 vs. 53, R1881 = 100 in rat cytosol). Unfortunately, when evaluated by Ali, *et al.*, the agent demonstrated little selectivity *in vivo* [23,34]. As a result, this compound was not examined for its ability to cause radiation induced cell death. Hochberg's group subsequently prepared a series of 7α-iodo (and fluoro) androgens as potential imaging agents. From this series, the radiohalogen was introduced by simple nucleophilic displacement into a steroid nucleus bearing appropriate 19/17α substituents [35,36]. They evaluated the effects of dihydro testosterone vs. dihydro nortestosterone vs. 17α-methyl dihydro(nor)testosterone. While the affinities compared to 5α-DHT were quite good

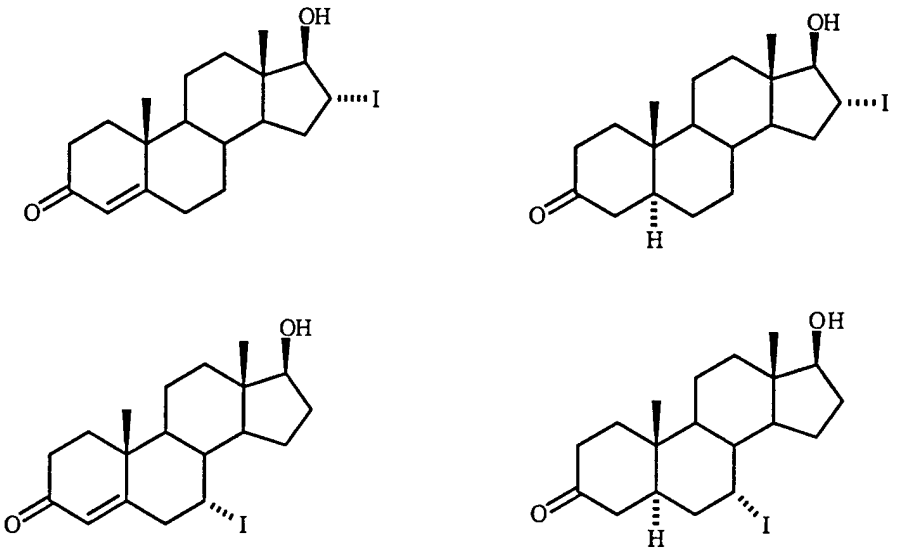


Fig. (8). radioiodinated (dihydro)testosterone derivatives.

(RBA = 25-123, DHT = 100) the radioiodinated agents were ineffective both *in vitro* and *in vivo*. As a result, no further work was pursued with those radiochemicals.

Radiolabeled antiandrogens constitute an even smaller series of potential therapeutic agents. This is due in part to the relatively small number of compounds that display this type of pharmacological activity. Until recently only flutamide, anandron and bicalutamide were the only agents approved as antiandrogens although newer nonsteroidal compounds are in clinical trials. (Fig. **9**). Miller and coworkers [37] reported the synthesis of radioiodinated bicalutamide via the triazene and trimethyltin intermediates. The iodinated derivative had affinity greater than the parent compound (3.1 nM vs. 11.0 nM), however, this was still poorer than testosterone (1.1 nM). In their I-125/123 labeled form this radiochemical may have potential as a radiotherapeutic agent, but no further data has been provided since the initial disclosures.

D. Summary

The past decade has seen advances in the synthesis of Auger-emitting ligands, both agonists and antagonists, for the steroid hormone receptors.

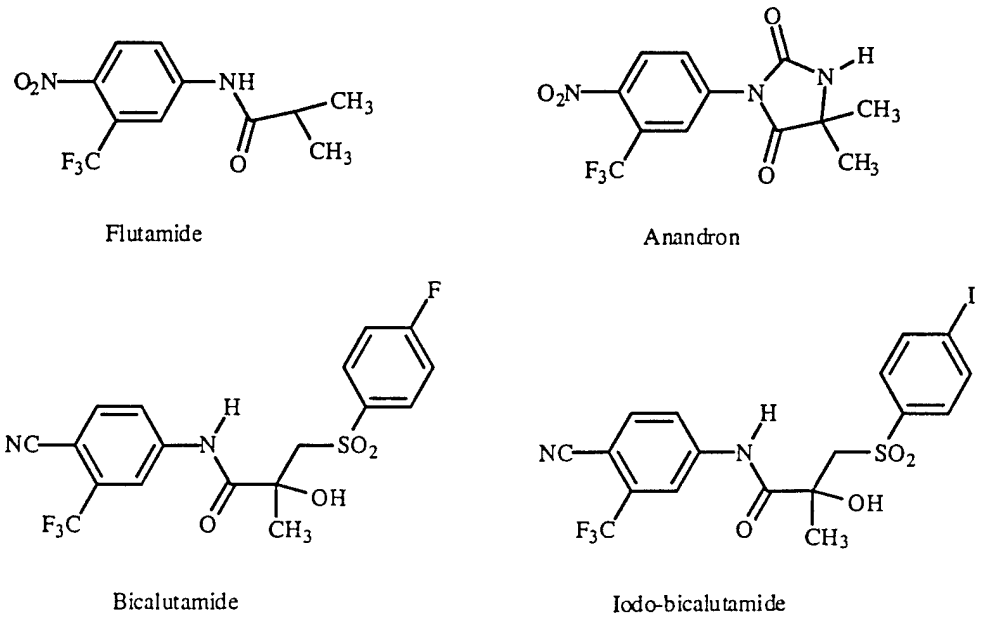


Fig. (9). Antiandrogens and radioiodinated analog.

Strategies have been developed for maintaining substantial affinity for the receptor and imparting metabolic stability in most cases. Use of the radioiododestannylation has been the most successful means for rapidity incorporating the radiohalogen in high specific activity. So far only the estrogen receptor-directed agents have demonstrated the ability to produce significant tumor cell killing. Successful extension to therapy remains to be shown for the estrogenic ligands. Improvements in receptor affinity and metabolic stability are required before the progesterone and androgen receptor directed agents can be evaluated as therapeutic agents.

III. DNA DIRECTED AGENTS

This section examines the work done over the past 10 years to develop agents that directly target the DNA. Deoxyribonucleosides (D nucleotides) and DNA intercalating agents constitute two other classes of compounds capable of imparting the cytotoxic effects of Auger-emitting radionuclides to the nuclear DNA. Labeled analogs of the deoxyribonucleotides can be incorporated into the DNA by the enzyme DNA polymerase if they resemble the endogenous substrate. This is one of the mechanisms by which antineoplastic drugs such as 6-mercaptopurine, 6-thioguanine, and adenine arabinoside, exert their cytotoxic effects. Appropriate nucleosides containing iodine or bromine could also be incorporated into the DNA and, upon disintegration, provide the low energy electron shower directly onto the DNA. Intercalating agents, on the other hand, are polycyclic compounds of either natural or synthetic origin that insert themselves between the bases of the DNA. Their ability to disrupt or to stabilize the structure of the DNA inhibits processes associated with DNA replication and ultimately exerts a cytotoxic affect. Auger-emitting analogs of the intercalating agents have the ability to induce strand breaks if the nuclear decay occurs during the time that the agent resides in the helix.

A. Radiolabeled Nucleoside Analogs

Among the nucleosides which could be applied to radiotherapy of tumors, halogenated analogs of uracil have been most extensively evaluated. This

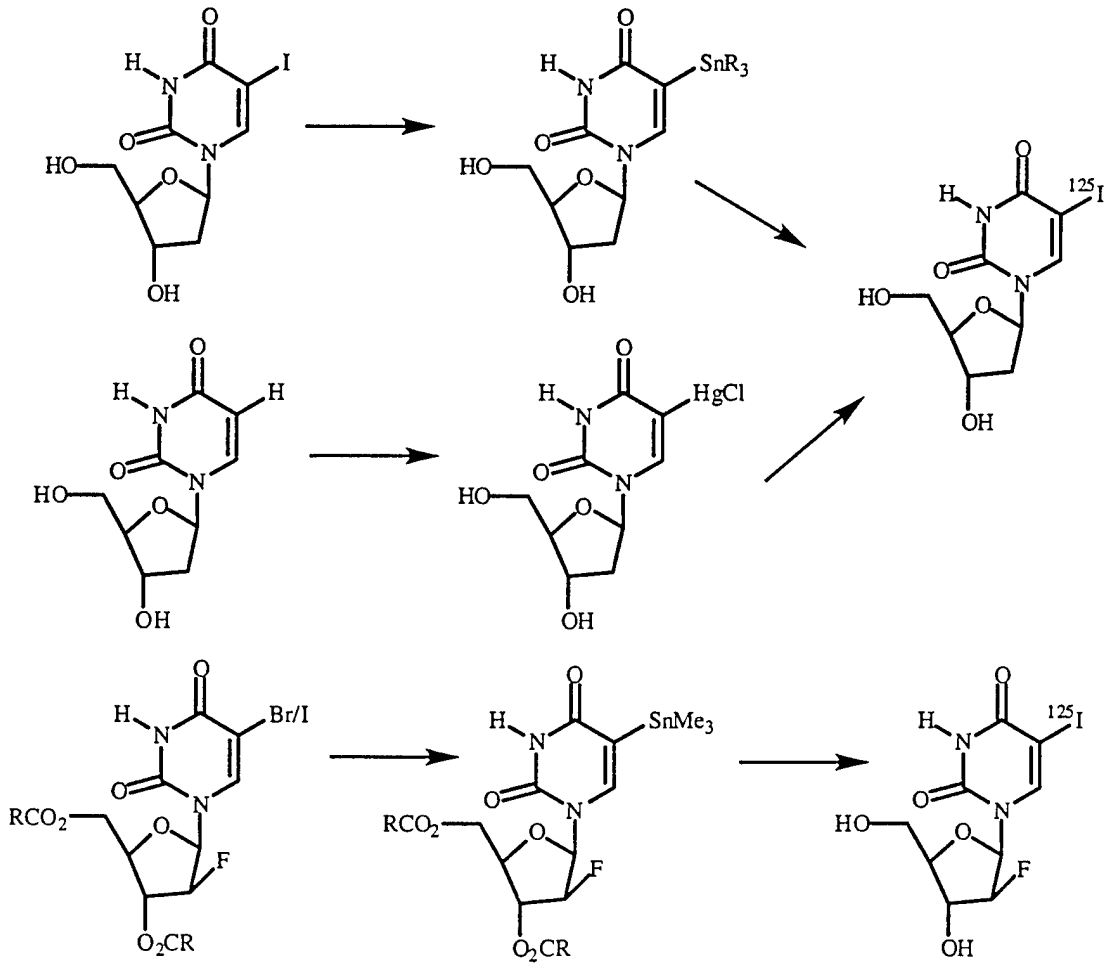


Fig. (10). representative syntheses of radioiodinated nucleosides.

emphasis is the result of earlier studies that suggested that 5-iodouracil in particular is a close structural analog of thymidine and that it substitutes for the natural pyrimidine base in many of the ribosylation and kinase reactions preceding incorporation into DNA. The two major strategies are the synthesis of the radiohalogenated derivatives that incorporate improvements in the radiohalogenation procedure itself and the synthesis of nucleosides with improved biological characteristics.

Among the examples of radiohalogenations of nucleosides or their derivatives, two that best illustrate the methodological improvements are the synthesis of iododeoxyuridine and its 2-deoxy-2-fluoro analog (Fig. **10**). The preparation of the former agent was reduced to a kit formulation by Foulon and Kassis [38,39]. In one method, they chloromercurated deoxyuridine to give the 5-chloromercuri-derivative which could be converted to the radioiodinated product using labeled iodide and Iodogen. The alternate procedure began with the cold iododeoxyuridine which was converted to the 5-trialkylstannyl intermediate with Pd(0) catalyst and hexaalkylditin. Radioiodination with iodide and hydrogen peroxide then gave the desired product. Both methods were virtually instantaneous, however, the demercuration method was more applicable to kit use. Vaidyanathan and Zalutsky [40] also employed the stannylation-destannylation method, however, their brominated or iodinated precursor required synthesis from the arabinoside and pyrimidine starting materials. The key iododestannylation step proceeded in greater than 85% yields to give the desired products.

The preparation of novel nucleosides/nucleotides is illustrated by two recent examples (Fig. **11**). Dougan,*et al.*, [41] began with iododeoxyuridine and following protection as the 5-Fmoc ester coupled it at the 5-position of the pyrimidine with bis(tributylstannyl)ethylene. Activation at the 3-position of the sugar with a phosphoramidate group allowed the intermediate to be incorporated into an oligonucleotide that was ultimately radioiodinated using [I-125]-iodide and various oxidants. Reed, *et al.*, [42] also prepared a radioiodinated oligonuleotide via iododestannylation . In their synthesis, however, they utilized a sequence that contained a terminal hexamethyleneamine to which a 4-tributylstannylbenzoyl moiety could be conjugated. Radioiodination used their standard method and the product was obtained in good yields and high purity. Although the investigators implied potential radiotherapeutic applications, no data were provided.

B.DNA Minor Groove Binding Agents

Another approach for the design of Auger-emitting DNA targeted agents involves labeling compounds that bind to the minor groove of the DNA via multiple hydrogen bonds. An example of the labeled intercalator method is illustrated by the synthesis and evaluation of [I-125]-iodoHoechst 33342 by Kassis and co-workers [43-46]. In their synthesis (Fig. **12**), it was necessary to choose a site which could simultaneously permit the insertion

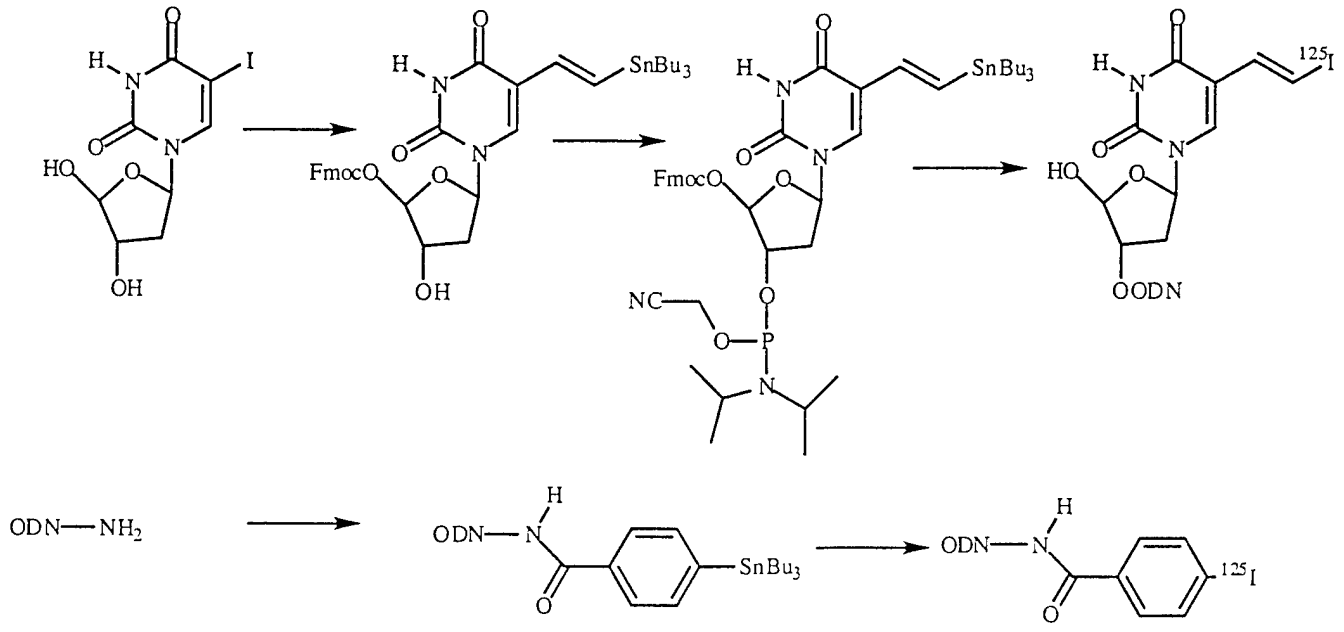


Fig. (11). Examples of Radioiodinated oligonucleotides.

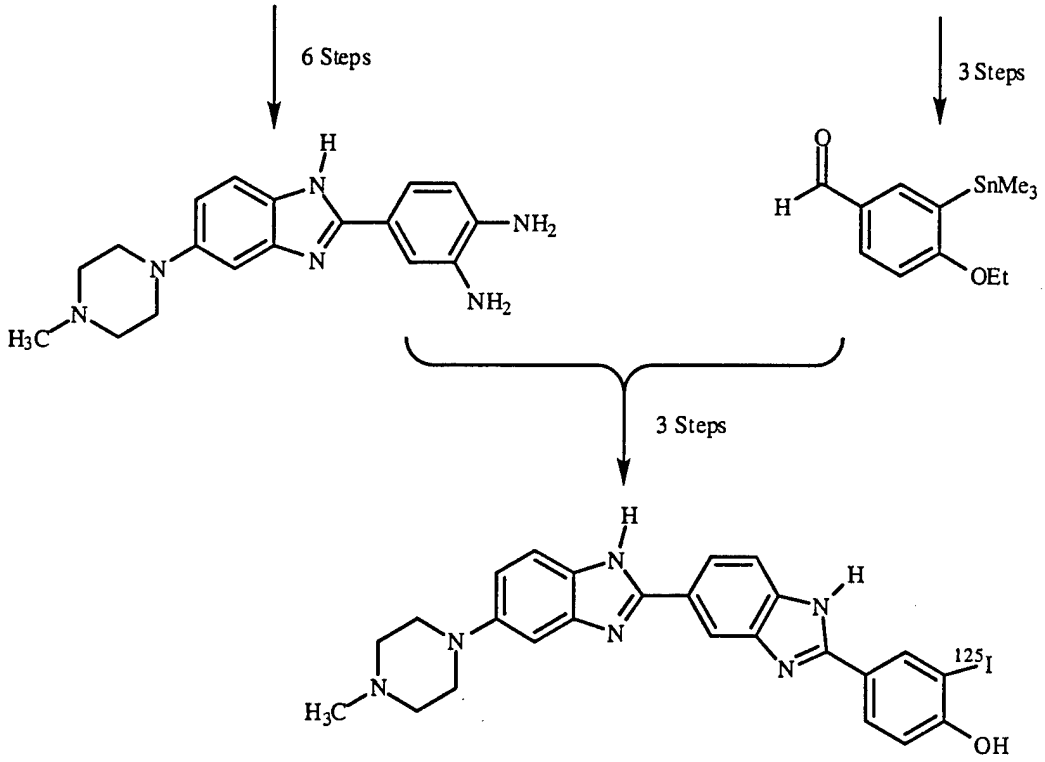


Fig. (12). Synthesis of radioiodinated iodoHoechst 33342.

of the trimethylstannyl group for radiolabeling while not adversely affecting the binding of the agent to the DNA. This was achieved by inserting an iodine on the distal aryl ring that could be replaced by the requisite stannyl moiety. With the availability of other sequence selective minor groove binding agents related to netropsin and distamycin [47] it should be possible to prepare and evaluate other Auger-emitting compounds as therapeutic agents. A relevant example is the modification of a sequence selective binder by Sigurdsson [48] to crosslink DNA. Replacement of the alkylating group by a labeled conjugate may achieve a comparable biological effect.

C. Summary

In the area of DNA targeted agents there has been modest progress in the field of radiosynthesis. While methods have been developed for the efficient preparation of labeled nucleosides, both for incorporation into DNA or into oligonucleotides that bind to the DNA, it is not clear whether the *in vivo* incorporation of the agents is sufficient to induce effective cytotoxicity. A similar problem may exist with the minor groove binding agents, however, the flexibility in their construction may ultimately lead to diagnostic or therapeutic agents.

IV OTHER SYNTHESIS OF AUGER ELECTRON-EMITTING AGENTS

Although the majority of radiosynthesis of (potentially) Auger electron-emitting agents have focused on the nuclear DNA as their ultimate target, studies on other approaches have also been reported. Radioiodinated antibodies with anticancer potential continue to be evaluated, with the utilization of Auger electrons perhaps as part of their mechanism of action. While most radioiodinations use the conventional electrophilic incorporation with an oxidant [49,50], others use the trialkylstannylaryl carboxylate NHS ester conjugating agent [51]. This latter procedure continues to generate interest, not only for its diagnostic potential but also for incorporating Auger electron-emitting radionuclides [52-56]. Since there have been some studies exploring the utility of Auger emissions as a therapeutic adjunct in the MIBG treatment of neuroblastoma [57,58] syntheses of other radiolabeled MIBG analogs have been reported [59]. Whether this is a viable approach to therapy remains to be seen. Lastly, the preparation of a somatostatin analog containing a chelated Auger electron-emitting radionuclide was described by Heppeler, *et al.* [60]. While little biological data were provided, its synthesis constituted one of the very few instances that did not employ a radiohalogen.

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reaction rate is sonication. Application of high energy ultrasound or power ultrasound to a solid-phase reaction has been found to accelerate the reaction rate as well as enhance the efficiency of resin washing. The talk will discuss the use of power ultrasound in a multiple, parallel synthesis format as a substitute for mixing, heating and washing paramagnetic supports involved in multistep solid-phase organic chemical reactions. Selective solid-phase examples from various combinatorial libraries will be presented.

8:35 Advanced Synthetic Techniques for Solid-Phase Synthesis

Dr. Randall Scheuerman, Affymax Research Institute

Solid-phase synthesis has afforded the ability to generate hundreds of thousands of compounds in screenable library formats. Using the available tools, as well as creating new tools, we have developed ways to quickly identify, optimize, and rehearse divergent chemistries compatible with solid-phase synthesis in the production of encoded libraries for screening. Topics to be discussed include:

- Chemistry selection criteria
- Novel chemistries on solid phase
- Library design issues
- Library production and quality control

9:05 Development of a Solid-Phase Synthesis Approach to the Preparation of Novel Steroid-Based Libraries

Dr. Robert N. Hanson, Department of Chemistry and Pharmaceutical Sciences, Northeastern University

Based upon the X-ray structure of the steroid hormone-ligand binding domain (LBD), we have projected several potential families of ligands with modified biological properties. Our approach involves the initial immobilization of a functionalized steroid to a carboxylated resin. Depending upon the nature of the terminal functionality, various directed libraries can be prepared by split synthesis. Cleavage from the resin generates discrete entities for screening as hormone receptor ligands.

9:35 Synthetic Approaches to Benzopyrone Combinatorial Libraries for Identification of Selective Bioactive Agents

Dr. Robert W. Brueggemeier

Molecules with a benzopyrone ring system include a number of natural products such as the bioactive flavonoids found widely in higher plants. Our initial medicinal chemistry efforts developed synthetic methods for constructing benzopyrones that are accomplished under mild reaction conditions allow for flexibility in substitutions and provide for moderate to high yields. Our novel synthetic route utilizes readily available salicylic acids and terminal alkynes as starting materials to construct the benzopyrone nucleus. Substituted salicylic acids are coupled with various terminal alkynes via a one-pot acid chlorination-Sonogashira coupling to give the desired alkynones in excellent yields. Conversion of the alkynones to enaminoketones and subsequent cyclization to the benzopyrone ring can be effected in a single step. Synthetic approaches for diversifying the benzopyrone skeleton have been developed, and solution-phase combinatorial chemistry is utilized to construct small flavonoid libraries.

10:05 Poster and Exhibit Viewing and Refreshment Break

11:00 ClickChem: Process Driven Discovery

Dr. Hartmuth Kolb, Chief Operating Officer, Coelacanth Corporation

Even a cursory glance at Nature's favorite molecules, reveals a striking preference for making carbon-heteroatom bonds over carbon-carbon bonds. Following nature's lead, we endeavor to rapidly generate substances by joining small building blocks together via heteroatom links (C–X–C), using a set of powerful, highly reliable and selective reactions. Click Chemistry is simultaneously defined, enabled, and constrained by the handful of nearly perfect, spring-loaded, reactions it depends on. The stringent criteria that a reaction must meet to approach Click Chemistry status will be discussed along with examples of the molecular frameworks that are easily made using this powerful, albeit limited synthetic strategy.

11:30 Metal Catalyzed Solid-phase Synthesis with Gaseous Reactants

Dr. Andrew Patron, Director of Chemistry, Charybdis Technologies, Inc.

Summary not available at time of printing.

12:00 Panel Discussion

12:30 Luncheon (sponsored by Cambridge Healthtech Institute)

BUILDING BLOCKS AND HIGH-THROUGHPUT LIBRARY PURIFICATION

1:45 Chair's Remarks

Dr. Nathan A. Collins, Director of Operations, Axys Advanced Technologies, Inc.

1:50 Biologically Derived Chemical Diversity: A Rational Approach to Library Production

Dr. Cheryl Garr, Director, Synthetic Discovery Chemistry, New Chemical Entities, Inc.

Natural products have historically been the feedstock of drug discovery. With the advent of high-throughput organic synthesis the emphasis has shifted to synthetics, usually at the sacrifice of overall chemical diversity inherent in natural products. New Chemical Entities, Inc. is focused on using automation, high-throughput technologies, and chemo/bio-informatics, in part developed for combinatorial chemistry, to advance natural products. Lead finding, optimization, and refinement can all be addressed competitively allowing for the capture of the quality in natural products and the quantity of HTOS. Examples of various techniques currently in use and under development will be provided.

2:20 ChemNavigator.com- Compound Selection Technology for Drug Discovery

Dr. Tad Hurst, Chief Technical Officer, ChemNavigator.com, San Diego, CA

ChemNavigator.com™ provides researchers with a secure system for Internet-based chemical exploration and procurement for optimizing biologically active compounds. The iResearch™ System combines customizable chemical design tools, the world,s largest searchable compound database, and various other sources of information to permit users to find and purchase compounds that are relevant to their research needs. ChemNavigator provides filters that present customized views of the available drug-like substances that are known to be available and are similar to a lead compound or are direct analogs of a lead compound. Other data related to the compounds can be used for selection, including expandability (a reflection of the known-neighbor count) and patent issues. Various technical considerations involved in this iResearch' System will be presented.

2:50 Intelligent Combinatorial Library Purification

Dr. Russell Scammell, Rhône Poulenc Rohrer Ltd. (tentative)

Summary not available at time of printing.

3:20 High-Throughput Purification of Chemical Libraries

Dr. William C. Ripka, Vice President and Chief Scientific Officer, Ontogen Corporation

An overview of Ontogen's experience in high-throughput purification of spatially diverse chemical libraries derived from parallel synthesis will be described. Application of state-of-the-art instrumentation resulting from integration of Ontogen's engineering and analytical chemistry efforts has permitted preparation and purification of single-compound per well libraries on a ten to forty thousand compound scale. Pre- and post-purification analytical data will be described. Purification methodology will be emphasized.

3:50 Poster and Exhibit Viewing and Refreshment Break

4:30 A Practical Approach to High-Throughput Purification of Combinatorial Libraries

Dr. Nathan A. Collins

As more companies utilize combinatorial chemistry libraries for their screening efforts, it is becoming more interesting to attempt to obtain these libraries at higher purity. There are many obstacles to successfully purifying combinatorial libraries, not the least of which is the inherent diversity and therefore physical properties of the members of a single library, and the time needed for the purification process and interpretation of the results for hundreds of thousands of compounds a year. This presentation will address our efforts toward the practical

and scaleable application of HPLC methods toward the purification of thousands of compounds per week.

5:00 LC-MS High-Throughput Purification of Combinatorial Libraries

Dr. Ruidan Chen, Hitachi Instruments, Inc. (tentative)
Summary not available at time of printing.

5:30 High-Throughput Purification and Analysis for Combinatorial Chemistry

Dr. Mark J. Gardner, Pfizer Central Research
The many reasons for chromatographic purification of compounds from combinatorial chemistry will be discussed. The development of a system to carry this out efficiently and some learning from our experience of using it to purify tens of thousands of single compounds will also be presented.

6:00 Panel Discussion

6:30 Close of Day Two

Friday, February 11

7:30am Poster and Exhibit Viewing and Light Continental Breakfast

SYSTEMS AND AUTOMATION

8:30 Chair's Remarks

Dr. Michael G. Organ, York University

8:35 Expediting Parallel Synthesis: Doing the Most Chemistry with the Least Effort

Dr. Michael G. Organ
Parallel synthesis strategies are being designed to facilitate as much bond-formation as possible, while minimizing reaction work-up and purification. This is being achieved through tandem reaction sequences utilizing common reaction elements (solvents, catalysts, etc.) and the incorporation of new equipment to facilitate multi-reaction sequences that require transfers and/or filtration. Other streamlining protocols such as on-resin and off-resin derivatizations are also being developed to maximize the chemistry while minimizing product handling.

9:05 Parallel Synthesis and Traditional Chemistry in Lead Optimization

Dr. Rolf Güller, President and Chief Scientific Officer, Chemspeed Ltd.
The predominant technology in combinatorial chemistry or parallel synthesis used for lead discovery currently is solid-phase synthesis. In lead optimization, solution-phase synthesis as well as synthesis on solid support is used, depending on the problem at hand. The majority of chemistry used in medicinal chemistry, process development, catalyst research, etc. has not been converted into solid-phase. Furthermore solution-phase synthesis is also the method of choice for producing novel building blocks and reagents as these are needed in larger amounts. Automating solution-phase synthesis on the other hand is more challenging. Ways in which this challenge was met in a medicinal chemistry project where automation was applied both for synthesis of building blocks and final, ready to screen compounds will be presented. Practical examples of the integrated approach (synthesis, work-up, purification, analysis and output) will be described, including the automated parallel synthesis of highly potent competitive and non-peptide inhibitors of aspartyl proteinases.

9:35 Novel Technology and Applications for Automated Ultrahigh-Throughput Organic Synthesis

Dr. Mark L. Peterson, Corporate Vice President, Advanced ChemTech, Inc.
Over the last decade, the tremendous impact made by the incorporation of combinatorial chemistry into discovery research programs in order to more rapidly and efficiently generate, analyze, test, and optimize target structures is difficult to match. Unfortunately, all currently available synthesis systems, critical to the actual construction of those target molecules, have some limitations in their operation or capabilities. In order to address these deficiencies and meet the varied needs of the diverse fields in which combina-

torial concepts are now finding themselves useful, a versatile and flexible instrument appropriate for applications from chemistry optimization to the generation of large libraries is required. Towards this end, the development and manufacture of the Venture Ultrahigh-Throughput Platform, which is capable of the fully automated, simultaneous execution of from hundreds to thousands of individual reactions has been completed. This was achieved through the integration of three new technologies: the Apollo reactor system, novel liquid handling robotics, and an operational software package. The particular features of this innovative instrument, its component technologies, and the advantages they provide will be discussed. In addition, specific chemistry investigations aimed at the synthesis of heterocyclic libraries, including benzodiazepines, thiazolidinones, dihydropyrimidines and 1,2,3,4-tetrahydro-b-carbolines, will be presented.

10:05 Poster and Exhibit Viewing and Refreshment Break

10:45 Parallel Chemistry Development, Library Synthesis, and Process Optimization

Dr. Alasdair A. MacDonald, Senior Manager, Chemistry & Support Services, Argonaut Technologies, Inc.

Today's chemist has increasingly come under pressure to make more compounds in a shorter amount or time. This necessitates the need to accelerate all aspects of the synthetic endeavor; from chemistry development to SAR exploration and library synthesis, to work-up and purification. This presentation will cover a range of traditional chemistries carried out on Argonaut's instrumentation. In addition, integration of various purification techniques on Argonaut's platforms greatly streamlines the synthetic process. Examples utilizing traditional liquid-liquid extraction (LLE), solid-phase extraction (SPE), resin-bound reagents/scavengers and solid liquid extraction (SLE) will be demonstrated to illustrate the benefits of such a parallel approach.

11:15 A New Platform for High-Throughput Synthesis

Dr. Ben Moshiri, Manager, Synthesis Products, Bohdan Automation, Inc.

A new synthesizer, developed by Bristol-Myers Squibb, was recently introduced to address a chemistry-productivity gap between fully automated synthesizers and small reaction blocks. The new platform allows combinatorial chemists to meet increasing throughput goals, while having the capability to carry out demanding chemistries in parallel. Recent modifications allow medicinal chemists to use the same platform for the synthesis of large quantities of compounds, in smaller arrays. A wide range of chemistry examples will be shown to demonstrate- solution- and solid-phase syntheses. This will include new work using novel linkers and resins developed by Prof. D. Enders' group at the University of Aachen, Germany. One example is the (-alkylation of SAMP hydrazones, requiring completely inert conditions at –70oC. Other chemistry examples include IRORI Kan™ cleavage and cyclization reactions, carried out to make 1000 to 5000 member libraries. An additional application discussed is the purification of reaction products using SPE.

11:45 Directed Sorting and Informatics: An Integrated Solution

Dr. David Chapman, President, Afferent Systems, Inc. and Mr. Barry Prom, Product Specialist, IRORI

IRORI's AccuTag system has gained wide acceptance in the drug discovery field and has become the de facto choice for library synthesis in numerous laboratories worldwide. In addition to performing synthesis, the synthetic organic chemist typically has to provide data on the libraries synthesized. Afferent Systems, Inc. has developed a suite of products that aid bench chemists and research IT staff in combinatorial chemistry. Afferent provides an integrated software solution for combinatorial chemistry informatics, including product structure generation, storage, and access; sample tracking and analytical information management; experiment planning and instrument control, all optionally using and Oracle-based enterprise-wide database. IRORI and Afferent have recently collaborated to develop a comprehensive interface for their two systems. The result is an integrated package for library synthesis and informatics. Capabilities of this system will be presented and the performance of this fully integrated system that combines library synthesis and laboratory informatics will be described.

12:15 Close of Conference